

CONTRIBUTION
TO THE
STRUCTURE AND DEVELOPMENT
OF THE
VERTEBRATE HEAD

A THESIS
FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY
PRESENTED TO THE
BIOLOGICAL FACULTY OF THE UNIVERSITY OF CHICAGO
NOVEMBER, 1894

BY
WILLIAM A. LOCY

Reprinted from JOURNAL OF MORPHOLOGY, Vol. XI, No. 3

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WILLIAM A. LOCY.

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GENERAL INTRODUCTION.

A GROWING interest has been manifested in the problems of cranial morphology as their importance in comparative anatomy has been more fully realized, and a number of strong investigators have taken up this particular field of study. Through their researches much has been accomplished; the views on cranial anatomy have gradually changed, as advance after advance has been made, until morphologists have come to look upon that wonderful complex—the head—not as a structure *sui generis*, but as the most extensively modified part of the animal, formed by differentiation and specialization from parts that are structurally homologous with those that follow in the trunk. According to this conception, the distinction between head region and trunk region is one of degree of differentiation and not one of kind.

The modifications which the head has undergone have been brought about gradually, and are so comprehensive in their range that if we could know their complete history, even in one animal, we should have a key to the leading questions of vertebrate phylogeny. But there are so many causes tending to modify the course of development, that we cannot depend on the steps of phylogenetic history being repeated in a complete and orderly way in any one animal form. Often a balance of probabilities must be struck to determine what is ancestral and what is secondarily acquired. The chain of evidence is indeed very incomplete, and must always be supplemented by a certain amount of inference; but what has not been fully enough recognized in the practical study of embryological development, is that the shortest intervals of time may be very important in keeping the connection. Coherency of the history must be preserved; and the difficulty of doing so is greatly increased by the fact that the new is made to proceed out of the old, and, frequently, one organ insidiously takes the place of an earlier formed one. Too great stress cannot be laid on the desirability of having a more complete series for study, and this is especially important in cranial anatomy. The traditional method has been to study one stage and then another "a little older," and to fill in the intervening gap with inferences. This has proved to be inadequate and misleading. It is now required that we shall have stages close enough together to trace the history of the transitory as well as the permanent organs. The practical difficulties in obtaining a sufficiently complete series are very great, and, in many cases, well-nigh impossible. Great effort has been expended in getting the material for the present research; it is a kind of material in which the stages cannot be controlled, and my series cannot be regarded in any sense as a complete one. Nevertheless there is represented in it several distinct stages that have not heretofore been described by students of elasmobranch embryology. Beard, in his study on the Transient Ganglion Cells and their nerves in *Rajabatis* says: "My series of the embryos of this form is what many might judge to be complete, numbering as it does some 300

specimens of all ages and sizes. Nevertheless there are gaps in the collection and these are often of a kind that it may not be easy to fill in. No two embryos are exactly alike in all the pictures which they yield of this apparatus, and in half a dozen specimens that would be taken to be of the same age from their sizes, from a comparison with Balfour's stages, or from the more certain criteria of number of gill-clefts, protovertebræ, etc., etc., it is quite common to find this transient nervous system, like other organs, in widely different stages of development and presenting great variations in detailed characters. In my researches on Raja I have been compelled to give up completely any attempt to make use of Balfour's nomenclature. With a limited number of embryos at one's disposal, it is easy to fit them into one or the other of the well-known stages; with an increased number this becomes more difficult or even impossible, so great are the variations met with." I have had a similar experience with my embryos of *Squalus acanthias* (*Acanthias vulgaris*). I am conscious there are gaps in the material, and, after making the best use of it that I can, I have, no doubt, missed many things not represented in my collection.

The studies recorded in this paper deal almost exclusively with the early history of the brain and sense-organs. Some of the questions upon which direct evidence is brought are: What was the primitive condition of the nervous system of the Vertebrates? What was the number and nature of the primitive neural segments entering into the brain? What has been, in general, the line of modification along which they have been converted into the brain? And what were the early steps in the differentiation of sense-organs?

I have been greatly indebted to Professor C. O. Whitman for courtesies both at Woods Holl Biological Laboratory and at the University of Chicago, and I have also to thank him for advice and suggestions on the subject-matter of this paper.¹

¹ Soon after the appearance of the Zieglers' paper (Beiträge zur Entwicklungsgeschichte von Torpedo: *Archiv für Mik. Anat.*, Bd. 39, Hft. 1, Jan. 1892) Dr. Whitman suggested to me that I should work over the same ground—the gastrulation and formation of the germinal layers—in one of our North American

In glancing over the views regarding cranial anatomy that have been held since the beginning of this century, we find a suggestive hint in the shift of opinion, as to the decidedly relative nature of all our views on morphological problems. There has been a distinct rise in the point of view, but scarcely any of the earlier problems are yet solved ; new ones have been added, but those that have been handed down have grown broader and increased in proportions as a mountain on nearer approach. There has been a gradual change in front, keeping pace with the advance of our knowledge and with the changes in our methods of interpretation. During that period the very soul has been breathed into the body of comparative morphology, and as a result, our interpretations are directed towards explaining anatomical structures in connection with their past development.

At the beginning of this century, on account of superficial appearances, the conception took rise that the head is divided into definite segments. The obvious division of the skull by sutures was gradually worked into the theory that the cranium represents a number of modified vertebræ ; and then began on the part of anatomists, the efforts to determine the number of these segments or vertebræ in the skull. Oken and Goethe formulated the theory which was taken up by that eminent anatomist Richard Owen, who lavished upon it an amount of attention and labor that was worthy of a more substantial theory, but the mistakes of that great man have proved of benefit to other morphologists.

This was the beginning of the idea that the head region represents a definite number of segments. Originally founded on external features of no segmental importance whatsoever, and not essential to the question which they served to introduce, the problem gradually widened and deepened and reached the essential parts connected with this segmental condition. The first conspicuous change of front came with the delivering of

species of *Elasmobranchii*. I collected material (*Galeus canis* and *Squalus acanthias*) for that purpose, and began studies along that line, but, finding new and undescribed conditions in the head region, my attention was gradually drawn off from the original purpose and directed towards cranial anatomy.

the Croonian Lectures, in 1869, by Huxley, in which he completely overthrew the vertebral theory of the skull, withdrawing attention from the superficial sutures in the cranium and directing it to the cranial nerves and branchiæ as bearing evidence to the segmentation of the head. Gegenbaur, in 1872, studied the cranial nerves especially in relation to the branchial clefts and reached the conclusion that there are nine segments represented in the head. Another distinct advance was made by Balfour, who first studied the segmental divisions of the mesoblast in the head of the Elasmobranchs, and identified by this means eight head-somites clearly represented. He also expressed the conviction that there were primitively a larger number of segments but, owing to extreme modifications of the head region, they are no longer clearly represented. This was at bottom the same problem, but it was now shifted upon organs that are truly segmental.

From the time of its discovery, this segmental division of the mesoblast in the head became a great favorite with morphologists in elucidating the problem of head segmentation. The mesoblastic divisions seemed, so far as the evidence went, to embody the most direct survivals of the original segmentation and, therefore, to be the most promising line along which to work out the problem. Valuable contributions have been made along this line since Balfour's time, by Marshall, Van Wijhe, Dohrn, Killian, Oppel, and others, and the myotomes of the head have continued to hold their high position in the minds of morphologists as the most significant remnants of the original segmentation.

Notwithstanding all these researches the original problem is still unsolved. There is no agreement as to the number or the nature of the primitive segments, and about the only point that may be regarded as settled beyond controversy is that the head and brain were primitively divided into segments.

Recently, there has been added as a factor in the discussion, observations on segmental divisions of the neural tube. Although such divisions have attracted the attention of several observers, their importance in the problem of cranial segmentation has not been appreciated. They have been regarded as

of secondary origin, depending on the segmentation of the mesoblast. Nevertheless, I hope to show that these segments are the first to appear in the head region, and that they are entitled to more serious attention on the part of anatomists, as representing the most primitive segmentation of the head of which any traces are preserved.

PART I.—METAMERISM OF THE HEAD.

I. BASIS FOR THE DISCUSSION.

The more recent discussions on the metamerism of the head are based upon segmental divisions as shown in (1) cranial nerves and branchial clefts, (2) mesoblastic head cavities and (3) segments of the neural tube.

The first-mentioned basis may now be set aside as involving too much conjecture. McClure has stated the objections to it as follows: "We have positive proof that the degeneration of certain branches has taken place. This being the case, we have every reason to assume that whole segmental nerves may have once existed, which have completely degenerated, leaving no trace whatever of their previous existence. If such be the case, the segments originally connected with these degenerated nerves must necessarily be overlooked, if the existing nerves are made use of as a means of determining the original number of segments.

"Furthermore, the vagrant changes in the position of some of the cranial nerves must necessarily cause confusion. For example, take the sixth nerve, which in the frog and tadpole stages is situated between the first and second roots of the ninth nerve, a position somewhat posterior to its place of origin. This remarkable shifting clearly shows not only what great changes in position the cranial nerves are capable of undergoing, but it also goes to prove that we can find no reliable means of determining the primitive segments by means of their connection with the exit of the existing cranial nerves. Beard in taking up this problem made use of an important series of sense-organs for which he has proposed the name of 'Branchial Sense Organs,' from their development from thickenings

of the epiblast over each branchial cleft. The dorsal branches of certain cranial nerves fuse with these epiblastic thickenings; the superficial part of the thickening giving rise to a branchial sense-organ, while the deeper portion becomes the ganglion of the dorsal root of the cranial nerve. This close relation which exists between the dorsal branches of the cranial nerves and their corresponding sense-organs is undoubtedly of segmental character. But this line of research is beset by a great difficulty, namely, that the degeneration of certain branchial sense-organs would, in time, involve the degeneration of their corresponding cranial nerves, and such degeneration has certainly taken place, in part or in whole, leaving in doubt the primitive segments with which they were connected."

The second and third points mentioned are more important clues to the metamerism of the head. Muscle and nerve are, physiologically, so fundamentally related that we should naturally expect some close correspondence between muscle segments and neural segments, and metamerism of the head region should be studied in light of the work done on both sets of structures.

The myotomes (or muscle segments) have received by far the most attention as they are the more conspicuous, but it is timely to ask whether they afford the most reliable evidence as to the primitive number of brain segments. Comparative study shows that the neural segments are the first to appear and are less subject to modifications than the muscle segments of the head. The large number of myotomes described in the head of selachian embryos by Dohrn and Killian are more transitory than the neural segments. The period in which they are exhibited is a short one, and soon the seventeen or eighteen segments of Killian, and the eighteen or nineteen of Dohrn, become reduced, by fusion, or absorption, or both, to the nine head segments of Van Wijhe.

The neural segments, on the other hand, begin very early, as shown in this paper, and preserve their original number and characteristics through several embryonic periods. It will be seen as we proceed in the account of these segments, that the assumption cannot be sustained, that the segmental divisions of the middle germ-layer (protovertebræ) are primitive.

II. HISTORICAL REVIEW OF THE WORK ON NEUROMERES.

The question of Metamerism of the Head as based upon myotomes has been completely reviewed by Dohrn, Killian, and others ; I shall say nothing on that side of the problem, but shall limit the historical review, and confine the discussion to the side of the question that has been less cultivated.

It is a fact of comparatively recent discovery that the whole neural tube of vertebrates is divided by constrictions into similar segments. Each segment is bounded, anteriorly and posteriorly, by transverse folds ; and the elevated area between them constitutes the segment to which the name metamere is given. These segments may be pictured to the mind as a series of transverse ridges and furrows occupying each side of the neural tube and not extending across the median plane. They are exhibited in very young embryos of Vertebrates and disappear before what may be called the middle embryonic period. The existence of such folds in the walls of the hind-brain has been known since the time of Von Baer, who in 1828, first observed them in the embryonic chick of the third day of development ; but it was not until 1889 that they were known to extend throughout the length of the neural tube.

Since Von Baer's time they have been observed and commented upon by various anatomists. Bischoff¹ figures the neural segments, but does not mention them either in the text or in the descriptions of the figures. His figures show seven folds in the region of the fourth ventricle of a dog embryo of the twenty-fifth day of development. There are also shown three additional folds in the region of the mid-brain.

Remak, in 1850, made important observations, and suggested that the segments in the hind-brain are connected with the origin of the nerves in that region. He noted five or six quadrilateral fields on each side of the hind-brain walls, calling attention to the fact that they correspond closely in position

¹ I am greatly indebted to Hoffmann's historical review of the literature in Bronn's *Klassen und Ordnungen des Thierreichs*. I have consulted nearly all the literature referred to there, but, in some few cases, where the original papers have been inaccessible, I have depended wholly upon his review of it.

with the visceral arches, and with the cranial nerves "which grow with them." According to his observations they fade away after the fifteenth day.

Dursy observed them in 1869, in the embryonic cow, of 6.5 mm. in length. He recorded the occurrence of six folds in the region of the fourth ventricle. Foster and Balfour, in 1874, noted the same structures in the chick, and suggested that they were of segmental importance. Dohrn, in 1875, called attention to the occurrence of eight or nine neural segments in the fourth ventricle of bony fishes. He contrasted this early segmentation with segmental divisions in insects. Götte figures such segments in the hind-brain of well-developed embryos of *Bomator*. In 1877, Mihalkovics was inclined to interpret these segments as due to mechanical pressure of the mesoblast, and, therefore, not a fundamental feature of the medullary tube.

Béraneck showed, in 1884, that there is a definite connection between certain of these segments and cranial nerves, thus giving the first real foundation for establishing their segmental relations. In his earlier paper, he describes five pairs of transverse folds in the hind-brain of embryos of *Lacerta agilis* from 3 to 4 mm. in length. He noticed that they rapidly fade away and disappear in embryos 5 or 6 mm. in length. In 1887, he studied the relations of these "replis médullaires" in the chick, and, as regards their connection with cranial nerves, reached similar conclusions.

Kupffer maintained in 1885 that these segments indicate a primary metamerism of the medullary tube. He has published several brief notices on these structures. In 1884, he gave a record of studies on the brain of the trout, in which he found five pairs of neural segments in the hind-brain. In sagittal sections he noted, in addition to these, three pairs in the mid-brain. He found no segments further forwards, and concluded that the fore-brain is not to be included in the segmented region.

In the following year ('85) he gave the results of his studies on embryos of *Salamandra atra*. In embryos of that form, showing as yet no traces of protovertebræ, he found

eight pairs of neural segments in the median part of the hind-brain. It is to be carefully noted that these segments observed by Kupffer were in embryos with a wide open neural groove, and occupied the median part of the cephalic plate, thus giving a "mediane Gliederung des Hirnes." In 1893, in his "Vergleichende Entwicklungsgeschichte des Kopfes der Kranioten," he gives figures (20*a* and 20*b*) of the forms described in 1885. These figures of *Salamandra atra* show segmental folds only in the median part of the neural plate, and none in the neural ridges. This is interesting when compared with my observations on amphibian eggs (see p. 529).

In addition to the eight segments in the brain region he counted thirteen or fourteen in the cord, extending backwards to a point a little in front of the blastopore.

Rabl ('85) speaks of unmistakable segmentation in the hind-brain of chick embryos of from fifty to ninety hours' incubation. He found seven or eight segments in the region of the fourth ventricle—not being able to determine definitely whether there were seven or eight. Again, in 1892, Rabl has most ably discussed the question of the metamerism of the head, but as his paper deals almost exclusively with segmentation in the mesoblast, it does not come in for attention in the present connection.

Oscar Hertwig gives the matter passing attention in the third ('88) edition of "*Lehrbuch der Entwicklungsgeschichte.*" He is not inclined to attach much importance to the neural segments.

Gegenbaur, also, does not look upon these particular segments as important factors in the metamerism of the head. His position on the question is shown by the following quotation so frequently met with: "So interessant und so vielversprechend diese Thatsachen sind, so wenig scheinen sie mir gegenwärtig geeignet, zur Beurtheilung der Metamerie des Kopfes selbst als Factoren in Geltung gebracht zu werden."

Orr, in 1887, traced very definitely the connection between these segments in the hind-brain and cranial nerves. In describing the segments he made use of the term "neuromeres," which has been generally adopted on this side of the Atlantic.

He describes six in the hind-brain of the lizard (*Anolis*), giving their anatomical characteristics with great clearness. He observed no neuromeres behind the point of origin of the tenth nerve, nor did he find them in the fore- and mid-brain, but he concluded, hypothetically, that they were present in the anterior brain regions. Orr found the fifth, seventh and eighth, ninth, and tenth nerves, respectively, connected with the first, third, fifth, and sixth neuromeres of the hind-brain.

Hoffmann, in Bronn's "*Klassen und Ordnungen des Thierreichs*" (1888), records his observations on these segments in *Lacerta* and *Tropidonotus*. He found seven in the hind-brain of these forms. In the following year he added further details in the *Zoologischer Anzeiger*. He differs somewhat from Orr as regards the relationships of the cranial nerves, assigning the fifth nerve to the second neuromere of the hind-brain, the seventh and eighth to the fourth, the ninth nerve to the sixth, and the tenth nerve to the seventh neuromere. From the first segment the fourth nerve arises, and subsequently shifts its position forwards.

McClure, following Orr's work, demonstrated the segmentation of the neural tube throughout its whole extent, and published a preliminary announcement of the same in 1889. He showed the presence in the spinal cord of segments continuous with those in the brain, and histologically similar to them. He examined these structures in the chicken, *Amblystoma*, and the lizard (*Anolis*). He fixed upon six in the chicken and lizard, and five in *Amblystoma*, as the number in the hind-brain of each respectively. He found two in the fore-brain, but left the number in the mid-brain undetermined, expressing the view, however, that there are two neuromeres in that brain region. Thus he identifies ten neuromeres in the entire brain region, and agrees with Orr in the assignment of nerves to the neuromeres of the hind-brain.

Miss Platt's work (1889), on "*Axial Segmentation of the Chicken*," agrees, in so far as neuromeric segmentation is concerned, with that of her predecessors, except as regards the relation of the nerve-fibres to their corresponding neuromeres. According to her observations they spring, primarily, from the

concavity between two segments, and not from the crest of a neuromere. My observations on the motor roots agree with those of Miss Platt in that particular.

Zimmerman ('91) states that he finds in embryos of the rabbit and chick, shortly before the closure of the neural groove, the segments observed by Kupffer in *Salamandra atra*. He noticed at first eight of these segments (encephalomeres) in the brain region. The three anterior ones were much larger than the five lying behind them in the medulla. The three front ones are the vesicles of the fore-, mid-, and hind-brains, and they straightway undergo secondary division as follows: The first divides into two, the second into three, and the third into three, making a total of eight secondary divisions arising from three primary ones. These added to the five of the medulla give a total of thirteen segments in the brain region. He also observed these structures in *Acanthias* and *Mustelus*, and found them very clearly defined. In mammals the metameres of the mid-brain are not so distinct. Zimmerman goes on to say that these folds cannot be accidental appearances, since in all classes of vertebrates corresponding nerves arise from corresponding segments. He gives a table, showing nerve relations, with too much detail to reproduce here.

Waters, whose complete paper appeared in June, 1892, studied especially the mid-brain of Teleosts. He confirmed and extended the observations of Orr and McClure. He counted eleven neuromeres in the entire brain region: six in the hind-brain, two in the mid-brain, and three in the fore-brain. He did not find neuromeres in the brain of the Cod earlier than the ninth day of development. He assigns the olfactory and optic nerves to the anterior two neuromeres. The two neuromeres of the mid-brain give origin to the third and fourth nerves, and from the six segments in the hind-brain the fifth, seventh, eighth, ninth, and tenth nerves arise as designated by McClure. The sixth nerve he found to occupy its theoretical position when the neuromere exists; when fusion has taken place between the trigeminus and abducens neuromeres the sixth nerve has been shifted backwards between the seventh and eighth nerves.

Froriep gave a noteworthy contribution to the subject of neuromeric segmentation in very early stages, before the Anatomische Gesellschaft of Germany, at the June meeting in 1892. He described anew the so-called neuromeres that he had previously observed in mole embryos, but concluded that they are not of true morphogenetic significance. He further described the conditions in Triton embryos, and concluded that the so-called primary neuromeres detected by Kupffer in those animals are simply the result of underlying mesoblastic somites.

Froriep agrees with Kupffer in finding segmental folds while the neural groove is widely open, and in locating them in the median part of the cephalic plate. But, whereas Kupffer finds eight in the brain region of *Salamandra atra*, he finds only four in the corresponding region of *Salamandra maculosa*, and five in *Triton cristatus*. (Compare with my observations, p. 529.) His general conclusion is that "the jointing of the vertebrate body is originally determined by the middle germ-layer; when ectodermal structures exhibit segmental arrangement, it is the result of secondary adaptation."

Herrick ('92), in a preliminary paper, gives an account of neuromeres in the Ophidian embryo, in stages after the complete closure of the neural groove, and after the formation of the ear vesicle. His figures show six neuromeres in the medulla. He states a proposition that will be of use later, in helping to distinguish between primary metamerism, and metamerism of secondary origin which show after the closure of the neural tube: "If neuromeres once existed in the fore-brain they would be visible only at an early stage . . . The so-called fore-brain neuromeres differ from those of the medulla and cord in involving only dorsal structures."

The present writer, in 1894, gave the first account connecting the earliest formed neuromeres with those of later stages. He showed that in sharks they arise very early, and may be traced without a break through all the stages of the open neural groove into the structures that have, in later periods, been designated neuromeres. The segmental divisions extend to the anterior tip of the fore-brain, and are distinguishable in that region for a brief time after the closure of the neural groove.

He also recorded the presence of neuromeres in *Amblystoma* embryos with wide open neural groove, but differs from Kupffer in locating them in the neural ridges instead of the median neural plate. (See further on this point, p. 530.)

From the foregoing historical survey it appears that our knowledge regarding metamerism in the neural tube has passed through the phase of simple observation of its occurrence (Von Baer, '28, Remak, '50, Dursy, '69, and others), and has grown by successive additions to the recent conception of its segmental importance. In reaching this point, first came the work of Béraneck ('84) and Orr ('87), showing that the neuromeres of the hind-brain are definitely connected with nerves; following this it was demonstrated by Kupffer, partly, in 1886, and by McClure, definitely, in 1889, that the neuromeres extend throughout the neural tube, and that those of the trunk region merge gradually into those of the head region. Lastly, the neuromeres of the mid-brain have been especially studied by Waters ('92), the condition of that brain region having been left undetermined by previous observers. Remak was the first (1850) to suggest the segmental relations of nerves and neural segments; Béraneck the first (1884) to demonstrate it. From this time onward the definite relation of nerves and neuromeres began to be studied, and both Orr ('87) and Hoffmann ('88) are pioneers in this line of study.

It will be observed that previous to the appearance of my paper just referred to, no one but Kupffer and Froriep had claimed a very early appearance for neural segments, and these two authors had recorded their appearance only in the median part of the neural plate, and not in the neural ridges. They had not shown the structures of the open neural groove stage to be in any way connected with the neuromeres of later periods, which are present in the lateral walls of the neural tube.

In the case of Froriep's observations I think there is reason to doubt whether the segments observed are really the "neural segments" of other writers. The small number (4 or 5) which he observed in the head region does not correspond with the number of neuromeres observed by any other author in the

same region. I think, also, there is a way to bring Froriep's observations into reconciliation with my own (see p. 529).

At all events, it has been understood from the work of previous observers that the neural segments arise after the neural groove is closed, or while it is in process of closing. Waters ('92) carries the idea throughout his paper that the metameric segmentation arises relatively late, especially in the Teleosts, where he was unable to find any traces of this segmentation earlier than the ninth day of development, after the auditory pit is formed. Orr and McClure do not in every case state ages, but from their figures and the text I understand that they have not detected this segmentation in very young stages. Miss Platt mentions the fact that these segments sometimes occur in the chick while the groove is open. Rabl mentions them as being especially clear from the fiftieth to the ninetieth hour of incubation in the chicken.

I have been fortunate enough to find these neural segments in a number of animal forms in extremely young stages, and in *Squalus acanthias*, to trace them coherently onward into the later stages. In this form, the division of the embryo into segments takes place before the neural groove is formed, and, before any protovertebræ have made their appearance, the metameres not only extend the whole length of the embryo, but they are continued for some distance into the embryonic rim. They occur under such conditions in this animal that they cannot be interpreted as depending on mesoblastic segmentation. In *Amblystoma* and the newt (*Diemyctylus*) the metameric segmentation is present in the rudiments of the neural folds, just after their first appearance and during their period of broadest expansion. In living chick embryos of about the twentieth hour of incubation they can be made out with clearness along the walls of the beginning neural folds. It is only in *Squalus acanthias*, however, that I have traced the complete history of these neuromeric segments.

III. DESCRIPTIONS OF STAGES OF ACANTHIAS.

The earliest stage in which I have detected this metameric segmentation is represented in Pl. XXVII, Fig. 25. This is an age somewhere between Balfour's stages *B* and *C*. It is, in reality, the youngest embryo of *Squalus* to which I have had access since I began to observe especially the metameric segments in that animal. Whether or not they occur in still younger embryos I do not know, but they are already clearly defined in the stage referred to, and it is reasonable to suppose that they may be seen in still earlier stages.

The axial embryo (Fig. 25) is just fairly established, and has reached a length of $1\frac{1}{10}$ mm. The head-end is already wider than the rest of the embryo. It has begun to show that tendency to broaden that is characteristic of the head-end of the embryo. The gastrular cavity is broad, and extends to the extreme anterior end of the embryo. In the figure it is seen even protruding beyond the head-plate. The primitive furrow, that has often been confused with the neural groove in these Elasmobranchs, is broadened at its anterior end. Fig. 63, Pl. XXIX, is a sketch of a horizontal section of this embryo to show the general appearance of the metameres in section.

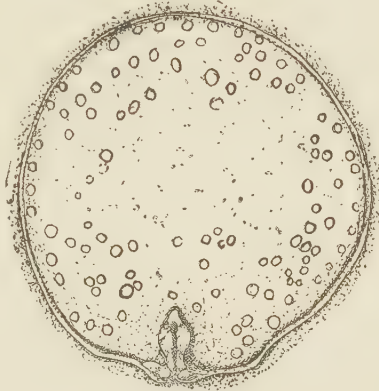
The segmental divisions in this embryo extend from the anterior end backwards along the margins of the axial part of the embryo, and out into the non-axial part or embryonic rim. There are seven or eight pairs of these segments in the embryo, and as many more, directly continuous with them, in the embryonic rim. The latter is segmented to the points where it is broken from the rest of the blastoderm. Whether or not these segments extend further into the blastodermic rim, I am unable to say.

The segments are most clearly defined along the inner margin of the embryonic rim, and extend more faintly across it. In the axial part of the embryo they are not in such a favorable position to be observed from above — they are on the rounded margins; but if the embryo be rolled into such a position that the margin is brought into view, its division into

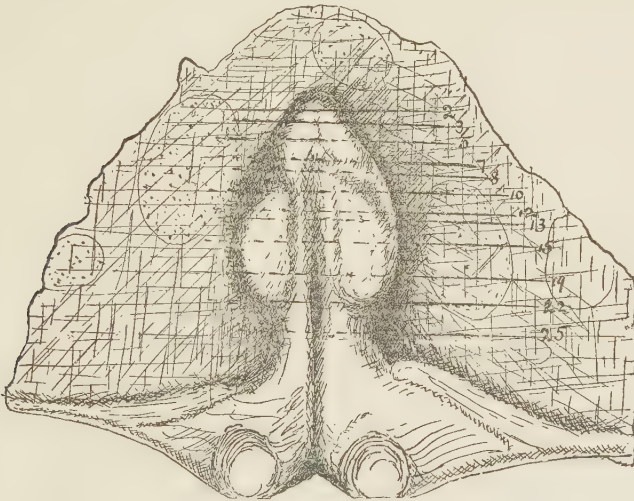
segments is more plainly seen. Near the middle part of the embryo the lines of segmentation are faintly traceable from the margins towards the median furrow. The two lines of segments are joined in front by a single median piece or segment. This unsegmented anterior tip becomes more prominent in the immediately following stages. There is no evidence to show whether this represents the primitive anterior segment or several aggregated anterior segments. These segments, once established in this very early stage, may be traced onward in an unbroken continuity until they become the neuromeres of other observers, and sustain definite relations to the spinal and cranial nerves. Ryder, in 1881, observed segmental divisions extending into the embryonic rim of *Elacate*, one of the Teleosts. In 1885, he figures such structures in a stage in which the neural groove is closed and the eye vesicles are well established. Although the figure shows a considerably later stage than we are now dealing with, and he does not speak of their earliest origin, nevertheless, the feature of their extending beyond the embryonic axis into the blastodermic rim agrees with my observations on *Acanthias*, and I think it not improbable, that Ryder's segments correspond with those I have described. These segments, observed by Ryder under such unusual conditions, have generally been interpreted by morphologists as due to precocious segmentation in the non-axial mesoderm. The segmentation I have just described is not capable of such interpretation, for sections show that the mesoderm is not yet divided into proto-vertebræ at this stage, and that the epiblast is the seat of the segmental divisions. The mesodermic somites of *Squalus* are formed later in the usual way, and the first ones appear in the trunk or neck region at a later period.

In Fig. 26 the embryo is relatively more slender in the trunk region, and there is coming to be an observable distinction between the broadly expanded cephalic plate and the narrower body. Upon the anterior end there is being formed a protruding unsegmented median tip, which is much better seen in Figs. 27 and 5. The median furrow ends in front in a broadly expanded depression. The gastrular cavity has become nar-

rowed in front by folding in of the sides, so that the embryo, when viewed directly from in front, seems mounted on a keel; the keel, however, is not solid, but contains the anterior part of the gastrular cavity, which still reaches to the anterior limit of the head. For the purpose of fixing the stages as definitely as possible, I give anatomical characteristics not immediately connected with the metameric segmentation. The embryo from which the figure was made measured $1\frac{9}{16}$ mm. in length, but there is so much individual variation in the size of embryos of apparently the same age, that the length is not very significant. There are, in that part of the embryo



CUT 1.—Embryo and blastoderm of *Acanthias* just before the formation of the neural folds. $\times$ about 8 diameters.



CUT 2.—The same embryo $\times$ about 40 diameters. Metameres not shown. The transverse lines and numbers indicate the plane of the sections shown in the succeeding cut.

behind the cephalic region, three or four mesodermic somites rather imperfectly differentiated. The metameric segmentation

is very clearly exhibited along the lateral margins of the neural plate, extending from the unsegmented tip backwards, and, as in Fig. 25, is continued in the embryonic rim to the points on either side of the latter, where it is broken from the rest of the blastoderm. While it is the lateral margins that are most clearly divided into segments, in the trunk region the lines of division may be traced inwards towards the median furrow. This is probably due to the appearance of the mesodermic

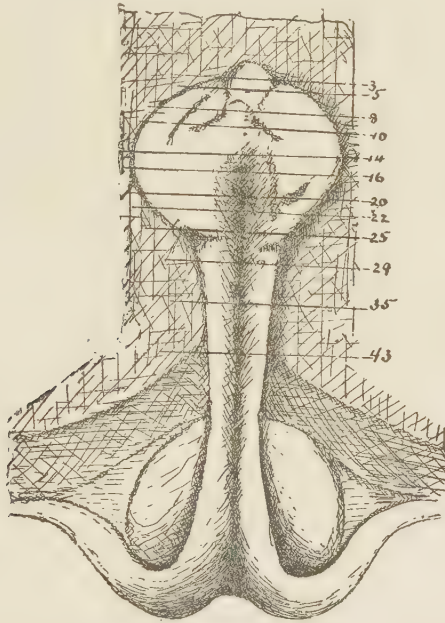


CUT 3.—Twelve transverse sections of the embryo figured in the preceding cut. $\times$ about 30 diameters. The numbers refer to the positions of the sections in the series.

somites in that region. Fig. 64 represents a horizontal section of this embryo showing metameres in the ectoblast.

Fig. 27 is in many respects similar to Fig. 26; it is slightly older and has reached a length of 2 mm. and shows about five mesodermic somites. Diverging furrows have appeared upon the cephalic plate that include between them a wedge-shaped central piece which terminates in the anterior unsegmented tip before mentioned. The cephalic plate is thus separated into a median and two lateral parts. It will also be noted in this figure that the lateral margins are marked off from the rest of the medullary plate by two furrows running lengthwise of the embryo, so that the plate is bordered, as it were, by marginal bands. The furrows are most distinct in the head region, but

they extend also, with less distinctness, into the trunk and fade away without reaching the hinder extremity. The furrows do not show so distinctly in cross-section as one would at first suppose, and they are in part an optical effect, arising from the way in which the neural folds are formed. This will be understood on reference to cut 5. There is, nevertheless, a distinct notch to be seen in the cross-sections of many specimens, while in others it is lacking. I am inclined, with my present light,

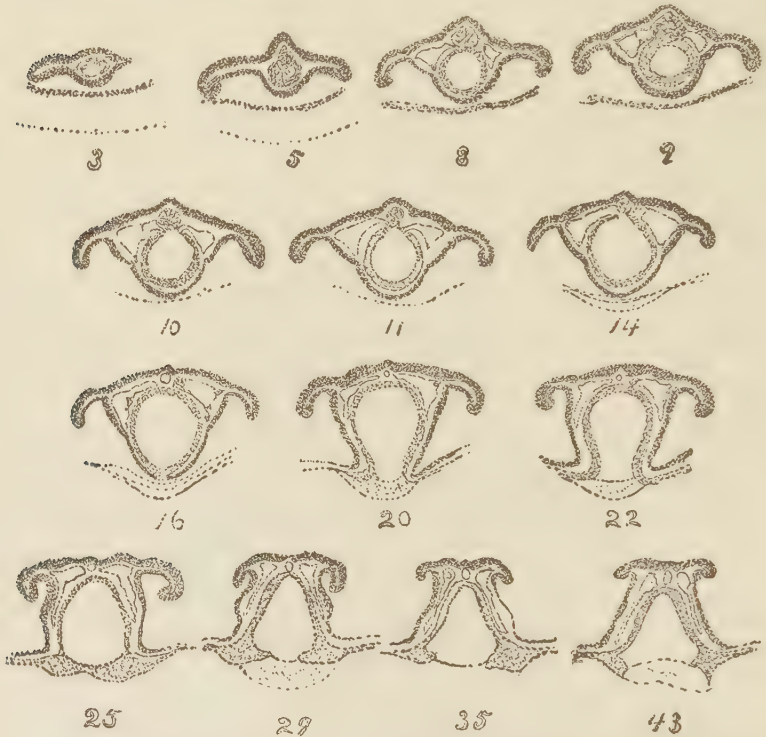


CUT 4. — Embryo of *Acanthias* just after the formation of the neural folds. $\times$ about 40 diameters. Metameres not shown. The transverse lines indicate the plane of the sections in cut 5.

to consider these furrows as purely mechanical effects. Sections show (cut 3) that the marginal bands, at a stage just younger than this one, are composed of an accumulation of cells forming thickened cords running along the margins of the embryo. These bundles of cells and their immediate derivatives are the material out of which the neural ridges and a large part of the medullary folds are straightway produced.

As noted in the preceding embryo, the metameres are most clearly seen from below, but the reason for this is not far to

seek. The condition of the neural folds in this animal is very unusual: when first formed they are lateral, wing-like expansions, extending along each side of the embryo, overhanging the yolk (cut 5). No sooner are they formed than they become ventrally curved, and, in this way the most clearly segmented



CUT 5.—Twelve transverse sections of the embryo of the preceding cut. $\times$ about 30 diameters.
The neural folds are expanded laterally beyond the body and ventrally curved.

parts of the embryo are brought ventralwards, and this accounts for the metameres being most distinct when viewed from the ventral surface.

Figs. 28 and 29 represent two views of the same embryo; it is an older stage than that represented in Fig. 27. The neural folds are now fully formed and their ventral curvature is very marked. In Fig. 28, the optic vesicles (*op*) are seen on each side of the central tongue-like process, to which attention has already been called.

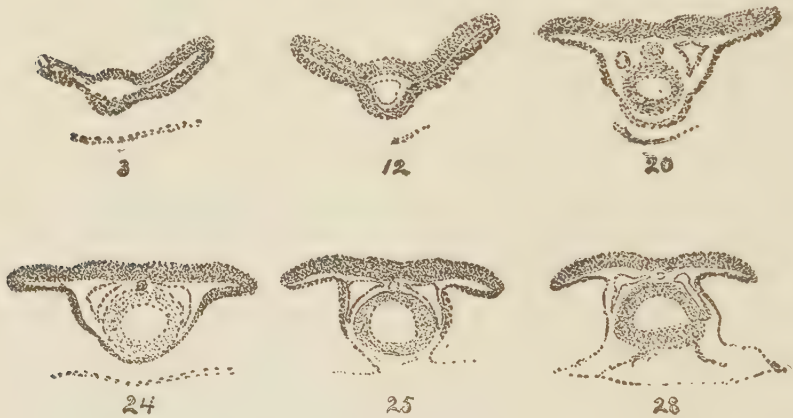
In Fig. 29,¹ the view is taken from below ; the embryo has been removed from the blastoderm and placed upon its dorsal surface and the recurved edges of the neural folds are thus brought prominently into view. This is of course the most favorable position for making observations. The dish containing the embryo should be placed over a black, non-reflecting background, and the embryo rotated into the most favorable position with a fine artist's brush.

In the actual specimen from which the figures were made the segments showed most beautifully. They appear like a row of beads running along the ventrally recurved margin, and extend with great distinctness the entire length of the embryo. Those in the trunk region are continuous with those in the head and pass into the latter without any transition forms. There is, however, some individual variation in size of the neuromeres, and they are not absolutely symmetrical on the right and left sides, but the significant thing is, there is uniformly the same number on each side in a given region, such as the hind-brain, or the brain region as a whole. Fig. 29 shows the central unsegmented piece from below with three segments on either side of it, occupying a part of the head-folds that is directed forwards. Following the beaded edge, from the head into the trunk region we find it disappearing from view beneath the expanded walls of the gastrular cavity. Viewing the same embryo from above (Fig. 28), the metameric segmentation is seen to extend the entire length of the embryo and, as in the earlier stages, laterally into its expanded parts. The segments are so plain that they may be easily counted. There seems now to be a natural landmark separating the cephalic plate from the rest of the embryo; this is an abrupt downward bending (*f*) in the medullary folds which, as I have determined, lies just in front of the future origin of the vagus nerve. There are eleven metameres in the lateral margins of the cephalic plate, including the ones embraced in this fold.

Fig. 30 represents an older stage, in which the medullary

¹ In the process of transferring and shading, the outlines in this figure have been rendered too symmetrical, giving to it a semi-diagrammatic character.

folds have unrolled from their ventrally curved position and are in the process of growing upwards. Those of the head are at this stage nearly in the horizontal plane (cut 6). The outer margins of the folds are plainly divided into segments, and the segmentation extends backwards, also, into the trunk region, but not so clearly defined. The optic vesicles are clearly seen on the head-plate, but the *accessory* optic vesicles (see p. 57) have not yet made their appearance. Beginning at the front end and counting backwards eleven segments on either side, we come to the point where the broadly expanded cephalic plate



CUT 6. — Six transverse sections of an embryo older than the preceding one. $\times$ about 30 diameters. The numbers below the sections refer to their position in the series. The neural folds are growing upwards and in section 24 have reached the horizontal plane. The depressions in Sections 3 and 12 are the optic vesicles. The embryo from which the sections are made is shown on Pl. XXVI, Fig. 7.

passes into the narrower neck and trunk. This, as before indicated, is the point of future origin of the vagus nerve. It seems to me to be a natural line of division which may be of service in determining the limits of the embryonic head. The question will be returned to on p. 543.

It should be borne in mind that all the stages so far described are very young; the earliest ones are before the formation of the embryonic medullary folds, and the oldest one is just when the medullary folds are arching upwards to form, for the first time, a medullary groove. The mesenchymic somites have, in the interim, appeared in the trunk region, and have

produced faint surface indications in the median parts of the medullary plate.

Taking a considerable step forwards in the history of these segments, we come to the condition represented in Fig. 31. This figure shows a stage in which the medullary folds have attained a nearly vertical position; they are about to bend towards each other and meet in the median plane, but, as yet, they have not become approximated in any part of their course, and, therefore, we have an open neural groove (see cut 9, p. 553) extending the whole length of the embryo. In this figure the embryo is viewed obliquely from the right side. The rudiments of several organs have now appeared upon the head; the most anterior of these organs is the primary optic vesicle; just back of this, near the margin of the head-folds, is seen a similar elevation that represents the combined vesicle of the mid-brain and the first *accessory* optic vesicle (see p. 556). Still further back in the same line, is another similar but smaller elevation, which, I think, represents the second accessory optic vesicle. Behind the latter structure the margin of the medullary fold is bent abruptly downwards; this is a normal condition in this stage, and is of course found in the earlier stages. Back of the primary optic vesicle and somewhat between it and the mid-brain vesicle, is a rounded eminence which is the external indication of the mandibular cavity, and behind this is the branchial pouch from which the branchiæ are subsequently formed. The front end of the gastrular cavity is being cut off along with the increase in the head flexure.

Directing our attention to the margin of the right medullary fold, we note that it is clearly segmented through the head region, and backwards into the trunk region, where, in the figure, it disappears behind the yolk. The metameres extend in reality to the posterior part of the embryo. There has been a slight change in position of the foremost segments with reference to the rest of the head-plate. The three anterior ones are no longer, as in Fig. 29, on a part of the margin that looks forwards, but they have been shifted backwards, and that part of the margin that was anterior, now constitutes a part of the lateral border. Of course this shifting of position is brought

about by changes in the medullary folds. The first three metameres are, at this stage, in front of the eye, the fourth, fifth, and part of the sixth, in front of the accessory optic and mid-brain vesicle. The following five segments (seven to eleven) occupy the reflected part of the neural fold. The eleventh, as has been indicated, lies in front of the vagus nerve.

The next stage to be considered (Pl. XXVII, Fig. 32) is just after the closure of the neural groove, which is, however, still open in front by a small neuropore. The original segments are still visible throughout the head region; those in the fore and mid-brains still show from the outside. The surface-markings on the head are similar to those in Fig. 31. In front is the conspicuous optic vesicle, and behind it is another similar rounded eminence, the mid-brain vesicle. Further back there is an area, circular in outline, resembling the mid-brain, except it is not so prominent. It is, however, merely a pad of mesoderm applied to the walls of the cerebellum. There is another surface indication of considerable interest, *viz.*, a line of mesoblast running over the branchial region; it connects in front with the mandibular cavity and behind with the body protovertebræ.

Fig. 33 shows a slightly older stage than Fig. 32. The neural groove is completely closed. The extreme anterior end of the gastrular cavity has been obliterated. The cranial flexure is quite marked. This is the last stage in which the metameres are visible throughout the length of the embryo; those in the fore- and mid-brain have become indistinguishable in surface views; they are, however, still to be detected in longitudinal sections. We possess now a particular advantage in dealing with these segments, because anatomical landmarks of the head regions have become established, and these enable us to say with definiteness what are the relations of the segments to the rest of the head. This is just prior to the appearance of the auditory vesicle; when first established its center occupies the space of the segment marked 10. Sometimes, in its earliest stages, the circular area spreads over the space of the three segments marked 9, 10, and 11, but I

should say from my observations that, more frequently, it is not so widely expanded. It always settles down in *Squalus acanthias*, to occupy the position first indicated, and, subsequently, it is shifted backwards. The topography of the head region is similar to what it was in Fig. 32. The chief differences to note are the further differentiation of the branchial arches and clefts; we may now distinguish the position of the future first visceral cleft, and, faintly outlined, the boundaries of branchial arches.

The segment marked 8 serves as an important landmark in all subsequent changes that affect the segments. It is seated above a depressed region in which the first visceral cleft subsequently appears, and, during all the time the segments are distinguishable from the outside, it has no nerve root.

Only a few words of description will be needed to enable us to follow the history of the metameres through the later stages. In Fig. 34, the auditory vesicle (*au*) has been formed and the first visceral cleft has broken through. The anterior metameres lying in front of the one marked 6 are no longer distinguishable from surface observation. The lines of neuromeres have been brought into contact in the median plane by the closing of the neural groove, but they are soon forced apart by the growth of the dorsal wall of the neural tube. We have now reached the stage, approximately, in which these neuromeres have been described by previous writers, — that is, the stage just after the appearance of the auditory vesicle, but it is to be remembered that Kupffer and Froriep have noted a form of segmentation in very early stages of *Amphibia* affecting the neural plate, but not the neural ridges (see p. 529).

In Fig. 35 some characteristic changes are to be noted; the auditory vesicle has shifted backwards till it occupies a position opposite the eleventh metamere; the metameres are being forced apart laterally by the growth of the dorsal wall of the hind-brain. The eighth metamere still serves as a landmark; there are now two clearly marked metameres in front of it and three behind it. The only metameres discernible from surface view are those belonging to the hind-brain. The mid-brain is considerably increased in expanse, and the first accessory

vesicle has been crowded forwards into the region of the thalamencephalon. This may be seen after the removal of the overlying layers of mesoderm, etc. (Pl. XXVIII, Fig. 44). The fifth nerve is already well begun, and nerve fibres are also given off from the segments numbered 9 and 10.

Fig. 36 shows an embryo slightly older than that in Fig. 35. The line of neuromeres have been forced further apart by the lateral growth of the dorsal wall of the hind-brain. The ear-vesicle is no longer circular in outline, but is fast becoming a closed pouch. The eye shows the beginning of the lens and the choroid fissure. The Anlagen of the fifth, seventh, eighth, ninth, and tenth nerves are distinctly visible from surface observation. The branchial arches are all clearly outlined and the first two gill-clefts have broken through. The specimen shows about forty-five mesodermic somites. A line of surface elevations over the hinder branchiæ mark the beginning of the lateral line.

In Fig. 37 the neural segments are undergoing some changes in outline that are likely to lead to confusion in identifying them in later stages. If, for example, we look along the lower margin of the segmented border, we shall see that the elevations and constrictions are substantially as they have been in all the previous stages, but those along the upper margin no longer correspond with them. In all the preceding figures the boundaries of the segments correspond on both upper and lower margins. In Fig. 37, however, the upper margin shows elevations just above the constrictions on the lower margin. These new-formed elevations become very quickly prominent, while the segments along the lower margin lose their individuality, and the segmented area becomes more and more an irregular sinuous band with crests upon its upper margin. The entire line of segments finally becomes indistinguishable, but if they be studied in stages immediately following that represented in Fig. 37 it will be the crests along the upper margin that first catch the eye. If the observations are made from above, these crests are seen to be transverse folds on each side of the medulla, and when counted will, of course, be one less than the original segments. It is only by viewing

them from the side, and comparing them with earlier stages, that we shall be able to identify the boundaries of the original segments.

Just what is taking place during the appearance of the crests is not now clear to me. I have heretofore assumed that it signified a union of the original segments, the anterior half of one with the posterior half of the segment lying just in front of it, but at present I am inclined to question that interpretation. The crests on the upper margin are between two neuromeres as designated by Orr, and they correspond in position to his inner ridge. The point of origin (motor fibres) of the fifth, seventh, and eighth nerves (so far as it may be determined by surface view) is now clear; they arise, as Miss Platt has described them in the chicken, from the concavity (on the lower margin) between two neuromeres. This will receive fuller consideration under the heading, *The Nerves*. It will be interesting to note incidentally, in this figure, the very large development of mid-brain over that in Figs. 33 and 34, and the consequent crowding forwards of the first accessory optic vesicle. The latter structure is also much reduced in size, and with its fellow is in the region of the thalamencephalon.

None of these figures have shown the condition of the entire neural tube. The segmentation so clearly seen in Figs. 34, 35, and 36, represents only a part of the actual segmentation, *viz.*, that in the uppermost part of the neural tube. The rest of the tube is too much covered by mesoblast to be seen without dissection. The upper part of the tube has — in the region of the hind-brain — two thickened lateral bands of cells, which form a border on either side of the neural tube; these are the segmented parts that are visible from surface observation. I have found it necessary to remove the overlying layer of mesoblast and the outer epidermic stratum, and completely expose the walls of the brain. When thus laid bare, the walls of the neural tube show in a most satisfactory manner. The ten or twelve figures following those just described show dissections of this kind.

Pl. XXVIII, Fig. 41, shows the surface view of an embryo slightly older than that represented in Fig. 33, and Fig. 42

shows the same embryo with the overlying tissues removed from the brain walls. It is clear from a comparison of these two figures that the line of metameres seen from the surface view are those occupying the upper part of the neural tube, and that below them the entire neural tube is divided into corresponding segments. The segments do not reach across the median plane, but they are alike in number and position on both sides of the tube.

Fig. 43 represents an embryo in which the auditory vesicle is just forming. The embryo was intermediate in age between those shown in Figs. 41 and 34.

Fig. 44 shows a dissection of an embryo of the same age as the one represented in surface view in Fig. 34.

Fig. 45 is taken from an embryo just younger than that represented in Fig. 35. By the growth of the dorsal wall of the hind-brain the line of metameres have been forced apart. This expanded dorsal wall, being a new growth, is not divided into segments.

Fig. 46 represents a slightly older embryo with the optic vesicle and also the auditory vesicle removed. There are in this figure eight segments in the hind-brain that show plainly, and faint indications of a ninth.

Fig. 47 represents a dissection of the embryo from which Fig. 35 was made, and therefore a direct comparison can be made between the two figures.

Fig. 48 shows the condition of the brain walls in an embryo just older than that represented in Fig. 37. From the continued growth of the dorsal wall the metameres on each side have become widely separated. The ear-capsule has not been removed.

Fig. 49 represents a slightly older embryo than the foregoing one. The auditory and the optic vesicle have been removed. There are now distinctly nine segments in the hind-brain region. The U-shaped segment, No. 12, in the hind-brain lies opposite the ear-capsule. This figure shows well the condition of the neuromeres described in Fig. 37, in which there is no longer (as in earlier stages) a correspondence between the ridges on the upper margin and those on the lower margin of the segmented lateral bands of the neural tube.

Up to this point the figures described have all been magnified uniformly 45 diameters; but on account of the increase in size of the embryos it will be better to carry forward the history of these segments with figures drawn on a smaller scale. Accordingly Figs. 50–60, inclusive, are magnified only ten diameters.

Fig. 50 represents an embryo of the same age as that shown in Fig. 32.

Fig. 51 shows the entire embryo, partly dissected, of which Fig. 43 is a portion more highly magnified. Behind the figure is seen the line of fusion of the lips of the blastopore.

Fig. 52 is a sketch of the embryo of which Fig. 47 is the enlarged view of a partial dissection. They all show well the segmented condition of the walls of the hind-brain.

Soon after the age represented in Fig. 55 is reached, the neural segments fade away. Figs. 57 and 60 (Pl. XXIX), represent the head region of older embryos in which the segments are no longer visible.

Taken together, the figures now described give a comparatively full view of the neural segments in different ages. They show them from their first appearance to the time they fade away. We learn from this examination that the neural segments are established before any embryonic organs appear, and that in the early stages they extend not only throughout the length of the embryo, but into the embryonic rim. In the earliest stages the segments are alike, and there is no structural distinction to be made between those in the head and those in the trunk, or even those in the embryonic rim.

In sagittal sections the neuromeres are well exhibited. Fig. 72 shows a section of a specimen just after the closure of the neural groove in which the five neuromeres belonging to the fore- and mid-brain are exhibited. The second neuromere coincides in the median plane with the neuropore. This is also to be seen in surface view in Fig. 32. Very soon the anterior brain regions become so much modified and expanded that the original segmental divisions are no longer visible.

Figs. 66–71 (Pl. XXIX), show six successive sections of a specimen slightly younger than the one just described. In

front are seen three of the brain vesicles, — those of the fore-brain, the mid-brain, and the cerebellum. The thalamencephalon and the prosencephalon do not show as separate parts. In the region of the hind-brain are seen three neuromeres especially well developed. They present the appearance of three bars; they are the seventh, eighth, and ninth neuromeres respectively. The other neuromeres are present, but they do not stand out with such distinctness as the three mentioned. When the ear vesicle first arises it makes its appearance opposite the ninth neuromere.

Figs. 73, 74, and 75 are sections of a somewhat older embryo after the ear vesicle is established. In these figures eight neuromeres of the hind-brain are visible. The ear vesicle is opposite the tenth neuromere. Just in front of it, in Figs. 73 and 74, are the roots of the eighth and seventh nerves, respectively, those from the former nerve being connected with the tenth neuromere, and those of the latter with the ninth neuromere.

In Fig. 75, the fifth nerve is seen to have connections with the first and second neuromeres of the hind-brain, *i.e.*, the sixth and seventh neuromeres respectively.

As already indicated, the eighth neuromere bears no nerve, and Hoffmann remarks, "This seems to be the case in all Vertebrates."

IV. SUPPLEMENTARY OBSERVATIONS ON OTHER ANIMALS.

I have also made some supplementary observations on these neural segments in *Amblystoma*, *Diemyctylus*, *Rana palustris*, *Torpedo ocellata*, and the chick. In all of these forms the metameric divisions are to be found in very early stages before any of the embryonic organs have been formed; and they are in all essential features like those I have described for *Squalus acanthias*.

Figs. 113 and 114 (Pl. XXX) show camera sketches of an *Amblystoma* egg, with broadly expanded neural plate and widely open neural groove. The neural folds or ridges are divided throughout their length into a series of segments with no espe-

cial distinguishing features between those of the head and those of the body region. The median plate included between the neural ridges is smooth at this stage; at a slightly later period, however, while the groove is still widely open, the median plate exhibits very faint transverse markings.

The contrast between this condition in *Amblystoma* and that in a closely related egg (*Rana palustris*) is very instructive. In the latter (Fig. 115) the cephalic plate is very obviously segmented, while the folds in the neural ridges are extremely difficult to see. We thus have in these two closely related eggs strikingly different conditions. In the one it is the median plate material that is thrown into obvious folds, while those in the neural ridges are well-nigh indiscernible; and in the other the conditions are reversed. This is not, however, to be taken as indicating profound differences; for a little careful observation shows that the median divisions do not correspond to those in the neural ridges, and therefore the median folds in *Rana palustris* are not to be compared to the primitive segmental folds in the neural ridges of *Amblystoma*. Careful observation shows, also, that there are segmental divisions in the neural ridges of *Rana palustris* that do correspond, in number and general characteristics, with those in the neural ridges of *Amblystoma*. These latter segmental divisions are extremely difficult to see in *Rana palustris*. There are four or five median transverse divisions in the cephalic plate of that form, while there are ten or eleven segments in the neural ridges of the same region.

These facts throw light upon the apparent discrepancy between the observations of Kupffer and Froriep and those I published in a preliminary article. Both the former authors observed segments in the median plate of amphibian embryos, and none in the neural ridges, while I have figured segments in the neural ridges of *Amblystoma*, and none in the median plate. But my later observations show that the appearances even in closely related eggs of the same age are not necessarily identical.

Froriep agrees with Kupffer as to the position of the segments in the early stages, but not as to their number. In

Salamandra maculosa he found only four segments in the head region, while Kupffer found eight in the same region of *Salamandra atra* (Fig. 117). All other observers have found eight or more in the head region, and Froriep stands alone in identifying so small a number of segments in the head. In *Rana palustris* there are about four obvious divisions in the median plate of the head (but, in addition, there are other segments in the neural ridges to the number of ten in the same region). This affords a suggestion that the segments observed by Froriep (see Fig. 116) correspond to those I have observed in the median plate of *Rana palustris*, and not to the segments in the neural ridges, which are evidently homologous with those in the same position in *Amblystoma*, *Acanthias*, and other forms.

Whether we find the median plate smooth in *Amblystoma* or faintly segmented depends upon the stage at which the examination is made, and we recognize that the appearances in any one egg are not constant throughout the open groove stage; and, further, that eggs of closely related animals are by no means necessarily similar at corresponding stages. The failure to take things of this nature (which are really common enough) into consideration has, I think, given origin to many differences of opinion and formed the basis of many a controversy. This should make us careful in comparing results to have the same material in precisely the same stages.

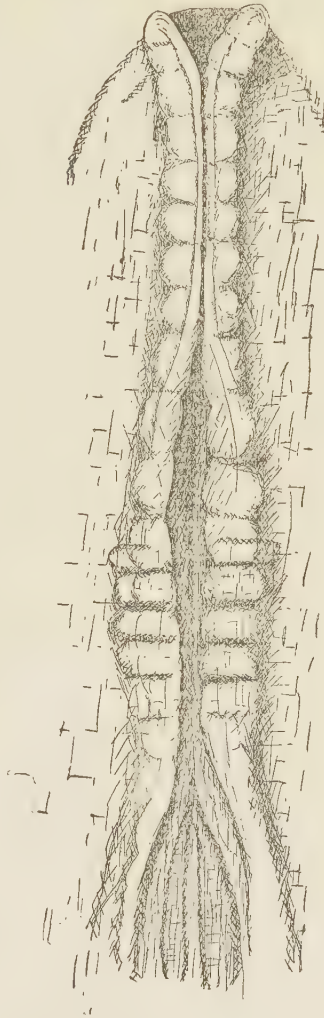
In *Diemyctylus* the conditions are very similar to those in *Amblystoma*. The neural folds show metameric divisions in the stages with wide-open neural groove. In the three amphibian forms I have examined there are about ten pairs of segments in the broadly expanded neural folds of the head.

In *Torpedo ocellata* I have also noted the occurrence of this metameric segmentation in several stages. The youngest embryos of that animal I have had corresponds to the stage designated "C" by the Zieglers, and beginning at that point I have traced it along through several stages with a widely open neural groove. This form is so closely related to the one I have especially studied that we would naturally expect—as is the case—marked similarity between the neural segments.

Torpedo is not so favorable for the study of the segments as *Acanthias*. The folds are much fainter in the former than in the latter, but the significant thing is, that the number in a given region in *Torpedo* corresponds to that in *Acanthias*.

In the chick these segments may be detected as soon as the neural folds are established, before there is any trace of protovertebræ. A small number is visible in the blastoderm of the twelfth hour of incubation just as the head-folds are first outlined. Their history has been carefully traced by one of my students, Mr. F. A. Hayner, and it agrees in all essential features with the history of the same segments in *Acanthias*. Cut 7 shows the appearance of these segments from surface view in a chick embryo of about twenty-four hours incubation. There are eleven segments in front of the first-formed protovertebræ. The cell-arrangement in the metameres, as shown in sections, is the same as that described by Orr in neuromeres of older stages of the lizard (*Anolis*).

From these supplementary observations it is clear that the occurrence of primitive neural segments, as the first definite expression of metamerism, is not an isolated case in *Squalus*. They occur in the same very early period in all the other forms examined, and in all of them precede any division of the mesoderm into protovertebræ.



CUT 7.—Embryo of chick with four mesodermic somites $\times$ about 45 diameters from a sketch by Fred. A. Hayner. The neural folds are divided throughout their entire length into primitive metameric segments, while there are but four mesodermic somites found.

V. GENERAL CONSIDERATIONS.

I. *New Aspect of the Problem of Metamerism.*

The neural segments have now been shown to occur in extremely young stages of a number of animals. The fact of their presence in these early stages once established they assume new importance. They have too definite a history to admit of being set aside as mere beadings or undulations of no metameric significance. When I first began, some two years ago, to notice these segments in extremely young embryos, I attached no particular significance to them; but a comprehensive study has convinced me of their segmental importance and everything taken into consideration they furnish, I think, a more satisfactory basis for interpretation of metamerism of the head than we have had before. I have pointed them out to several observers, in *Amblystoma* and the chick, and have found no one who had ever noticed them before; but through the kindness of others I have had my observations as to the number in the head region confirmed. Since these structures have been overlooked in the earliest stages, it will not be out of place to say a word about their general appearance, and how to see them.

It is extremely difficult to represent them on paper just as they look. All my drawings are camera tracings, and so far as number and arrangement of the segments are concerned, have been verified and reverified over and over again. They are a little too distinct in some of my drawings; in making the figures clear enough so there shall be no doubt as to what I mean, their distinctness has been somewhat exaggerated. On Pl. XXVI I have given a few photographs from untouched negatives that show these segments more as they appear in the actual specimens. To observe them successfully is largely a question of getting shadows. One can look directly at the crenated margins without seeing the segments at all, but by tilting and rotating the specimens until the proper angle of illumination is found, they may be seen with more or less distinctness, according to the general state of preparation of the

material. I have never seen a shark embryo, of the proper age, of any method of preparation, in which I could not detect them. A dead-black background is, of course, the best surface for observing anything of this kind by reflected light. A white surface is occasionally recommended, but it is desirable to cut off all reflected rays except those coming from the specimen, and one may see delicate structures on a black surface that cannot be seen at all on a white background.

The care with which the specimens are prepared makes a great difference in the clearness with which these structures may be seen. The conditions under which most material is hardened are unfavorable. There is usually an albuminous fluid in contact with the embryo, and this, together with minute fragments of yolk, coagulates when the fixing reagent is used, and forms a coating over the embryo. The embryos should be washed by a very gentle jet of the reagent immediately after their immersion, and the clouded reagent should be removed and replaced by clear fluid. This makes the preparations beautifully clear.

2. *Are they Artifacts?*

There is one question that must be answered for all new structures, *viz.*, are they artifacts — produced by the reagents used? Too great precaution cannot be taken in considering this question.

I have used the following reagents in preparing my material: picro-sulphuric acid, picro-nitric acid, Flemming's stronger solution, Davidoff's corrosive-acetic, chromic acid with a trace of osmic, corrosive sublimate removed with iodine. I have observed *Acanthias* material prepared by all these various methods, and in every case have found the segments as I have described them. I have always taken the precaution to count the number in the head region, and uniformly have found the same number of segments there, regardless of the hardening reagent used. I have had a complete parallel series of specimens hardened with Davidoff's corrosive-acetic and Kleinenberg's picro-sulphuric, and have compared the two, step by step,

one set serving to fully corroborate the observations made on the other.

In *Acanthias* I have followed the history of these segments very carefully, and have traced the earliest-formed ones *without a break* into the later stages, and identified them with the neuromeres. If, therefore, the segmental folds of the open neural groove stage are artifacts, it may with equal force be claimed that the so-called neuromeres, which are later stages of the same segments, are also artifacts.

It should also be borne in mind that similar segments exist in correspondingly early stages in *Amblystoma*, *Rana palustris*, *Diemyctylus*, and the chick. It is truly interesting to observe the way in which these different kinds of material bear out the interpretation, that we are dealing with veritable anatomical structures.

The most satisfactory reply to be given to the question is based on study of fresh material. Fortunately, the chick offers at all times a source where we can get living embryonic material at any desired age. I have repeatedly observed these segments in living chick embryos¹ in the eighteen to twenty-two-hour stages, and have treated them with reagents while they were actually under observation. The effect of the addition of picro-sulphuric acid is to render immediately the walls of the neural groove opaque and more clearly defined, but not to affect the number or arrangement of the segments. Camera sketches have been made before the addition of the reagent and compared with those made after the specimens were hardened. The two correspond, as regards number and arrangement of the segments. I have also noted these structures in living embryos between the twelfth and fifteenth hours of incubation. Their consecutive history has been traced in my laboratory by one of my students, and the results may be expected to appear later in this JOURNAL.

I have likewise observed them in living embryos of *Amblys-*

¹ The embryo was removed with a part of the blastoderm to normal salt solution, which was kept at the proper temperature, and the specimens tilted and rotated with a very fine sable brush over a black background. The structures are, of course, faint, and delicately, although definitely, outlined.

toma in the open neural groove stage and have compared them with hardened specimens of the same age.

3. *Do they afford a Clew to the Metamerism of the Brain?*

If we grant that these are truly segmental structures, the question still remains : Do they afford the best or even a good clew to the number of segments in the primitive brain ? If so, they must be shown to be equally important in this direction with myotomes, branchiæ and cranial nerves.

In estimating the claims of these various forms of segmental divisions, to rank as primitive, the question of the time of their appearance in developmental history will be significant. On this point I wish to observe, that in all forms I have studied — embracing representatives of Birds, Amphibia, and Selachians — the neural segments are among the first anatomical structures to be established ; before the vestiges of any organs have appeared, the embryo is divided throughout its length into similar segments. These metameric divisions, therefore, antedate myotomes, branchiæ, cranial nerves, or any other structures that exhibit metamerism. They persist through the early stages of development, and become definitely related to segmental nerves and segmental sense-organs. In the light of their very early appearance and their history, I think we are justified in saying that they are the most satisfactory traces of primitive metamerism that is presented in the group of Vertebrates. They may be looked upon as a survival of that general segmentation, which all agree in assuming for the (not too remote) ancestral form.

It should also be observed that the entire embryo is segmented, and the term "Metamerism of the Head" should be understood to signify merely regional metamerism and not a different kind of segmental division from that occurring in the rest of the embryo.

The study of sections shows that the neural segments are distinctly differentiated groups of cells, and not merely a series of undulations. As has been already pointed out the arrangement of cells is like that described by Orr for the neuromeres in *Anolis*.

4. *They are formed Independently of Mesodermic Influence.*

The next point I wish to maintain with regard to these segments is that they are formed independently of mesodermic influence.

In a preliminary account, I went so far as to state that the segmentation is epiblastic, but that statement should be qualified. It is certainly most clearly expressed in the epiblast, but the other germ-layers apparently feel the same influence, and partake of this segmental division in a modified degree. It is least clear in the mesoderm — in sections it may be made out in this layer, and also in the endoderm. The so-called neural segments are developed throughout the entire length of the embryo before any protovertebræ are formed and, therefore, they must be independent of any formative influence of the latter.

There is also a notable difference in the two forms of metameric division ; the formation of protovertebræ, as is well known, proceeds from a certain point forwards and backwards, and if the segmentation I have described were moulded over the former, we should expect the neural segments to appear gradually, keeping pace with the formation of protovertebræ.

Nevertheless, the majority of authors who have touched upon the question have taken the position that segmental division of the mesoderm is primary and that the neural segments are moulded over it, but all this time the early history of the neural segments has not been known. Kupffer announced, in 1885, that segments appear upon the median neural plate of *Salamandra atra* while the groove is widely open and before the appearance of any protovertebræ. He designated this primary metamerism. Seven years later, Froriep ('92) studied the question of metameric segmentation upon closely related forms (*Salamandra maculosa* and *Triton cristatus*), but reached different conclusions as regards the nature of the segments.

Froriep's conclusions are based on both surface study and sections. He shows in very young *Triton* embryos a segmented condition in the neural plate included between the

incipient neural folds. He expressly states that the segmental divisions do not extend beyond the plate into the neural ridges. The latter, he says, become segmented later ; only, when in the process of closing of the neural groove, they are brought directly over the originally segmented plate, and are made to feel the effect of the underlying segmented mesoblast. The number of segments which he detects in the neural plate at this period is very small. He finds only four in the greatly expanded anterior part.

My observations (already given on p. 530), differ from his in a fundamental way: showing, in *Amblystoma*, *Rana palustris* and the newt, the presence of unmistakable segments, in the earliest formed neural ridges, and not less than ten pairs in the broadly expanded head-plate.

In *Squalus acanthias*, also, it is the incipient neural folds that are the most plainly segmented, and in this animal they are so situated that they cannot possibly come under the influence of the mesoblast in any mechanical way. It will be remembered from descriptions of Figs. 27 to 30 and from sections of the same (cuts 3, 5, 6) that the neural folds are formed as wing-like expansions, extending laterally beyond the body. The mesoblast does not accompany these outfoldings of the epiblast, and when they best exhibit the segmental divisions no mesoblast enters into them.

As for the rest, Froriep gives figures of sections to show that the mesoblast is at this very early period actually divided into somites that correspond to the external segments. His figures certainly show segmental divisions, but being wood-cuts they are not entirely satisfactory when the question arises whether they are really protovertebræ. Froriep interprets them as such and closes his article as follows : "Die Gliederung des Wirbeltierkörpers ist ursprünglich an das mittlere Keimblatt gebunden ; wo sich als Produkte des äusseren Keimblattes segmentale Anordnungen finden, sind dieselben durch Anpassung an die Metamerie des Mesoblastes sekundär entstanden."

The internal condition figured by Froriep is interesting when compared with that in *Squalus acanthias* at corresponding stages. I have found in that animal an undulated condition of

the mesoblast, but it is not clear in my mind that we are justified in looking upon the divisions as primitive mesoblastic somites ; before seeing Froriep's paper I had considered them as undulations, probably due to the same influence that has thrown the epiblast so clearly into segments. In many embryos of *Squalus*, showing epiblastic segmentations, they are, so far as I can determine from sections, entirely lacking. It seems to me that they are rather to be looked upon as a consequence of primitive segmentation and not as protovertebræ. This view is substantiated by the fact that the formation of the latter takes place in the usual manner at a later period.

Considering the late stages in which the neuromeres have generally been described, it was a natural inference that they arise after the neural tube is established. Minot, in expressing his conception of the formation of the neural segments, based on the descriptions of Orr and McClure, says : " Their appearance seems to depend on the development of the primitive segments of the mesothelium. When the segments are fully formed, and before their inner wall has changed into mesenchymal tissue, they press against the medullary tube and oppose its enlargement ; at least one sees that the tube becomes slightly constricted between each pair of segments and slightly enlarged opposite each inter-segmental space." But the neural segments appear so much earlier than the primitive segments of the mesothelium, that this interpretation is no longer tenable.

The facts made known will tend to materially modify the current theory of metamerism, which assumes as fundamental that metameric divisions begin in and depend on the mesoblast. As Adam Sedgwick says, in a recent paper : " The segmentation which obviously persists in the trunk region, and which begins with segmentation of the mesoderm, and is moulded upon it in the manner characteristic of all metamerically segmented animals." It seems to me a strained inference, that the middle layer—the last germ-layer to be formed—should be the bearer of this primitive metameric division, but, of course, the final appeal must be made to sections and my sectioned material has given evidence entirely corroborative of

the surface observations ; sections of the embryo illustrated in Fig. 25 show that the mesoblast is nowhere divided into segments, while the epiblastic somites extend throughout its entire length. When the stage represented in Fig. 26 is reached, the first mesoblastic somites (three in number) are to be detected, in the trunk region ; the rest of the mesoblast remains undivided, but the epiblastic segmentation is as before. I have also made a careful study of the sections of the chicken embryo illustrated in cut 7, and can find no segmental divisions of the mesoblast outside the protovertebræ (five in number), but the metameric divisions of the epiblast extend from the extreme anterior tip back to the point where the neural folds fade out.

5. *Presence of these Segments in Embryonic Rim and Primitive Groove.*

Another point to be noted regarding these primitive segments is their range. They do not end at the posterior limit of the axial embryo, but extend on either side into the embryonic rim. As the embryo increases in length, those in the embryonic rim are apparently drawn into the axial embryo ; at any rate, I do not find evidence of extension by budding in the axial embryo, and, in the early stages, the number of neural segments therein increases in direct ratio to the lengthening of the embryo. This would indicate that at least some of the material of the embryonic rim contributes to the elongation of the embryo.

In the chick the walls of the primitive groove are also divided into segments that are similar to those that appear in the neural folds.

6. *Number of Segments represented in the Brain.*

There is considerable variance in the conclusion of different observers as to the number of neural segments represented in the brain. Orr, McClure, Waters, agree in identifying six in the hind-brain of the chick and lizard, and five in the hind-brain of *Amblystoma*. In estimating the entire number in

the brain, there is to be added, according to McClure's count, four for the combined fore- and mid-brain, and, according to Waters', five for these regions. Thus, McClure considers the brain to represent a total of ten neuromeric segments, and Waters makes a total of eleven segments.

The European investigators, in general, have found a larger number of these segments in the hind-brain, and usually have not observed them in the fore-brain. Dohrn gives eight in the bony fishes, Rabl seven or eight in the chick, Hoffmann seven in Reptilia; Froriep counted twelve in the entire brain of mole embryos, and Zimmerman gives thirteen in front of the vagus. The fore-part of the brain has been regarded as non-metameric, and the question of the anterior extension of segmentation is an important one. In regard to that point, the evidence furnished by the neural segments, shows that the segmentation extends throughout the fore-brain. This corresponds with the investigations of Whitman on the nervous system of Clepsine, in which he shows conclusively that the cerebral ganglia of that animal belong to the metameric series.

Recent observations on a different set of segments, *viz.*, segmental divisions in the mesoderm of the head, have brought to light a relatively large number of cephalic segments. Dohrn, in 1890, observed eighteen or nineteen mesodermic segments in the head of *Torpedo marmorata* of 3 mm. in length. Killian ('91) in the following year describes seventeen or eighteen in Balfour stages *F* and *J* of *Torpedo ocellata*. He also makes a correction to Dohrn's enumeration, making it correspond with his own. In later stages of Selachians, as Van Wijhe has shown, they are reduced to nine, or to ten according to Miss Platt.

There is such a fundamental relation between muscle and nerve that we might expect myotomes and neural segments to bear a direct numerical relation. My observations show a smaller number of actual segments in the brain-walls than that arrived at by Dohrn and Killian through studying the myotomes of the head region. In the hind-brain I have found eight segments represented in the earlier stages, and a ninth segment which, to all appearances, belongs to those of the spinal cord, but later this ninth segment becomes clearly associated

with those of the hind-brain. I have taken this to signify that the hind-brain has encroached on the territory of the spinal cord, and has embraced at least one segment originally belonging to the cord. It is reasonable, on theoretical grounds, to suppose that such a process has taken place and has been several times repeated.

In order to bring the segments of the brain into satisfactory evidence I have completely laid bare the brain-walls by removing the overlying layers of mesoderm and epidermis. The segments after being exposed in this way stand out so clearly that I feel considerable confidence in my count of them in *Squalus acanthias*. In addition to the nine segments in the hind-brain, I have found five in the combined fore- and mid-brain. In the latter particular I agree with Waters, but, of course, differ as regards the total number in the brain. McClure says (p. 39): "I will show that Dr. Hoffmann is probably wrong in considering the hind-brain as consisting of seven segments, and that the segment considered by him as the first segment of the hind-brain is rather the posterior segment of the mid-brain; in other words, it is the second neuromere of the mid-brain." The recently adduced evidence is all in favor of Hoffmann's observation. According to my observations, there are still represented in the otogeny of *Squalus* at least fourteen paired neural segments belonging to the brain region. There should be added to this enumeration the median unsegmented tip which terminates the line of segments in front, and, as stated above, there are reasons to suspect (although no direct evidence in that structure) that there may be more than one segment included in that terminal piece. If the terminal piece represents a single pair consolidated, then there are fifteen neural segments in the earliest condition of the brain of *Acanthias*.

7. Relation of the Neuromeres to Sense-Organs and Cranial Nerves.

The sense-organs and cranial nerves, undoubtedly, at first sustained definite segmental relations to the neural segments. In the spinal cord there is still a pair of nerves for each neuro-

mere, but in the brain the primitive relations have been greatly modified or obliterated. There is not sufficient evidence to show what might have been the primitive arrangement in that region. Of course, we bear in mind that the sensory fibres grow centripetally, but if they appertained to a particular segment, we might make a tentative estimate of numerical relations as follows :—

- I. First Neuomere of Fore-brain, Olfactory.
- II. Second Neuomere of Fore-brain, Optic.
- III. Third Neuomere of Fore-brain, possibly nerve to Pineal Sense-organ?
- IV. First Neuomere of Mid-brain, Oculo-motor.
- V. Second Neuomere of Mid-brain, Trochlearis.

It is easier to assign relations for the segments of the hind-brain. In my assignment I agree substantially with Hoffmann.

- VI. First Neuomere of Hind-brain, anterior root of Trigemini.
- VII. Main root of Trigemini.
- VIII. Third Neuomere of Hind-brain; no nerve root, at least in early stages.
- IX. Facialis.
- X. Auditory. The roots of the Facialis and the Auditory arise separately in *Squalus acanthias*.
- XI. Glosso-pharyngeal.
- XII, XIII, XIV. Roots of the Vagus.

At the time of its first appearance the auditory saucer lies in front of the tenth neuomere, but is soon shifted backwards opposite the eleventh.

Minot has directed attention to the fact that Miss Platt and McClure ignored the difference between ganglionic and medullary nerve-fibres, and this is important; but the ganglion ridges are divided in the same way as the neural tube, and we may speak of the neuomeres as including the segments of the ganglion ridge.

The relation of the nerve-fibres to the neuomeres will be considered more in detail under the heading of The Nerves.

8. *Head and Trunk.*

It would be a great convenience to anatomists to have some means of distinguishing between the head and trunk of very young embryos. It is generally regarded as impossible, on account of the lack of definite landmarks, to assign such a line of division, in early stages, before the origin of the auditory vesicle. As Sedgwick says, in the article referred to above, "The term head here must be regarded as meaning the anterior end of the body, for it is not possible in these young embryos to distinguish the head from the trunk." Nevertheless, in the young embryos of *Squalus acanthias*, there seems to be a natural line of division. The neural folds in this animal run forwards with the margins nearly parallel to one another, and then expand in front into the broad cephalic plate. The expansion takes place rather abruptly, and it is possible, in very young stages, to draw a line indicating where the expanded part of the cephalic plate joins the non-expanded part of the embryo. This line may be drawn without any reference to the number of primitive segments that it will cut off. The position of such a line is indicated by *AA* in Fig. 26. This is, in *Squalus acanthias*, just in front of the point where, subsequently, the vagus nerve begins. As before indicated, there are uniformly eleven neural segments in front of this line. Their number remains the same from the earliest stages until the anatomical landmarks (auditory vesicle and nerves) appear that enable us to determine the limits of the head region. In this animal, we may identify that part of the head that lies in front of the vagus nerve by counting the first eleven neural segments. It will be merely a question of agreeing upon the number of primitive segments belonging to the vagus, to enable us to locate with definiteness the hindermost limit of the head. Besides being of use in other ways, this would enable us to say, even in the earliest stages, what is head-mesoblast and what is trunk-mesoblast.

It is interesting, in this connection, to notice that there is in *Amblystoma* a similar greatly expanded cephalic part with an assignable line of its union with the non-expanded trunk

region, and there are also in this form ten or eleven segments included in the expanded head part. In the chick there are eleven segments in front of the first formed protovertebræ.

9. *Summary.*

Some of the more important facts and conclusions regarding the neural segment may be briefly stated:

1. They are natural structures, not artifacts produced by the reagents. This has been shown by their constancy in appearance and general characteristics when different reagents are used; their consecutive history; their similarity in different kinds of embryos; and lastly, by their presence in fresh material before any reagents have been used.

2. *Early appearance.* — The so-called neuromeric segmentation is the first to appear. It arises long before there are any segmental divisions of the mesoderm, and therefore cannot depend upon segmental divisions of the middle germ-layer. Neuromeric segmentation is more primitive than mesodermic segmentation.

3. *Structure.* — The cells in these segments are characteristically arranged even in the earliest stages, and their arrangement and their structure would indicate that they are definite differentiations of cell areas, not merely mechanical undulations.

4. *Extent.* — The entire embryo is divided into similar segments, and passes through an arthromeric condition similar to that of arthropods and worms. In *Squalus* the neural segments extend also into the germ-ring, and in the chick at times into the primitive streak.

5. *Number.* — There are in *Squalus acanthias* eleven segments in the brain region in front of the vagus nerve, and fourteen paired segments in the entire brain region, as follows: nine in the hind-brain, two in the mid-brain, and three in the fore-brain; this does not include the anterior tip, which may represent several consolidated segments.

6. *Backward growth.* — There is some evidence to show that the spinal cord region is being encroached upon by backward

differentiation. Early in the history of these segments there are seven that clearly belong to the hind-brain, and later there are successively added two more.

7. *Nature, position.*—These segments are clearest in the epiblast; the other layers are slightly affected by the segmental influence; the mesoblast least of all. The segments are serially homologous.

8. *Relations.*—They are directly (if not genetically) related to the cranial and spinal nerves. The neural ridges are divided in the same manner as the neural tube. The segments are also directly related to the sense-organs through nerves.

9. *Transformations or modifications.*—The modifications are most extreme in the anterior part with the early obliteration of those belonging to the fore- and mid-brains. Those in the hind-brain region are clearly defined for some time after the establishment of the cranial nerves, and then they fade away.

This is about all that can be said about their transformations, for the modifications have not yet been worked out in detail.

PART II. — THE SENSE-ORGANS.

The sense-organs of Vertebrates embrace all those structures that are endowed with special sensibility. They differ from one another mainly in degree of differentiation, and form a series, at the lower end of which are simple sensory papillæ, and at the upper end are complex organs like the eye and ear. We recognize two orders of sense-organs — simple generalized ones, the ganglionic sense-organs, and more specialized ones belonging to the so-called five senses.

The question of the origin and relationship of these sense-organs is full of interest. From the combined results of investigations on both Invertebrates and Vertebrates it seems altogether probable that the higher sense-organs have been derived from those of a lower order, and, indeed, that they have all been differentiated from a common sensory basis, and are therefore related in a direct way.

This view has been entertained by morphologists for upwards of ten years, and has a firm foothold in philosophical discussions. The evidence favoring such an interpretation has been accumulating, and although still incomplete, the hypothesis was never so well supported as at present.

Professor Whitman's researches were among the first to bring out this conception. Ten years ago he demonstrated in leeches the genetic relation between eyes and tactile papillæ, and since that time it has no longer been a matter of pure assumption to say that certain end-organs are modifications of a common sensory basis. He shows that the sensory papillæ located on each body-ring in the leeches have a very interesting relation. In the hinder part of the body the papillæ are purely tactile, but passing headward they become gradually modified, and are at first mixed visual and tactile, and finally the anterior ten pairs are purely visual. We have thus had for upwards of ten years a well-authenticated case of the derivation of sense-organs of a higher grade from those of a lower order.

In a more recent publication, "The Metamerism of *Clepsine*," 1892, Professor Whitman has added to the definiteness of his earlier discoveries by observations on a new species, *viz.*, *Clepsine hollensis*. He shows by a comprehensive study of the elements of the nervous system, the nerve distribution, and the sensillæ the complete homodynamy of all the segments.

Regarding the relationship of the eyes to other metameric sense-organs, he says: "One more peculiarity of this species may be noticed. The median rows of sensillæ are quite well developed as eyes, at least on segments IV, V, and VI. The visual elements in segment IV are quite numerous, and they are placed in a pigment cup quite like that of the principal eyes, only thinner and smaller, and directed backward instead of forward. The sensilla of segment V is a little smaller, but still presents the same general features. In segment VI the organ is still eye-like, but its visual elements are fewer and the pigment cup imperfectly represented by loose pigment. In segment VII we find one or two visual cells and a little scattered pigment. In the following segments we usually find the

sensillæ in about the same condition. In no other species hitherto described do we find the sensillæ passing by such gradations into the principal eyes. *The serial homology of these organs with the eyes is, then, a fact demonstrated not only by the embryological development, but also by the structural gradations exhibited in the adult animal*" (pp. 391, 392).

The existence of a series of rudimentary sense-organs in Vertebrates was brought to light in 1885 by Froriep and Beard. While it is by no means clear that they are the homologues of the sensory papillæ of annelids, it is nevertheless probable. It is now generally admitted that they constitute the basis from which the higher sense-organs of Vertebrates are derived. These organs are arranged in longitudinal series. The series has become rudimentary or lost in the adult forms of higher existing Vertebrates, but they are present in the embryos. Kupffer and others have shown that there are at least two series of these organs, the upper one corresponding to the lateral line of comparative anatomy, and the lower one embracing the so-called branchial sense-organs of Beard. From the upper series the ears and nose are probably derived, and possibly the eyes.

Allis ('88), in his well-known memoir on the lateral line of *Amia*, gives figures that show a connection between the epithelium of the nasal pit and that of the lateral line. His Fig. 1, Pl. XXX, representing a larval form one day old, shows the nasal epithelium forming part of the lateral line. In subsequent growth it follows the same course that the surface organs of the lateral system do when they become canal organs. Allis says, p. 537: "The nasal pits are inclosed in the same way that the lateral canals are, and the short canal so formed is at first continuous with the canal inclosing organ 5 infra-orbital."

There is now general agreement that the ears belong to the lateral line series, but there has been much dissent on the part of some morphologists to admitting the eyes to the same category; but the grounds for the opposition seem to me to be largely removed. It must be said, moreover, that the organs of taste and touch have not as yet been shown to have any genetic relation to the ganglionic sense-organs.

I am glad to acknowledge my indebtedness to Minot's introductory remarks to his chapter on the Sense-Organs. He says: "Very suggestive in this connection are the observations of H. V. Wilson ('91), of a thickening of the nervous layer of the epidermis on either side of the head in the bass embryo (*Serranus atrarius*). This thickening forms a long, shallow furrow, which subsequently divides into three parts, of which the first becomes a sense-organ over the gill-cleft, the second, the auditory invagination, and the third, the Anlage of the sense-organs of the lateral line. This peculiar development confirms the notion that all these organs belong in one series, but the appearance of a continuous thickening as the Anlage of them all has, as yet, been observed only in this fish, and may not indicate a corresponding ancestral condition. Unfortunately, Wilson was unable to make out anything as to the connection of the sensory plate with the ganglia. The sense-organ above the gill-cleft, although differentiated, is a larval structure only, and disappears in the adult."

A quite similar condition is now known to obtain in some elasmobranch forms. Mitrophanow, in 1890, published a preliminary report of his observations on the lateral line of *Acanthias* and other Elasmobranchs. In 1893 he published a full report of the same, illustrated by many figures. He describes a continuous thickening of the epidermis along the sides of the head embracing the territory of the roots of the seventh to tenth nerves. From this thickening there is separated the material for the auditory saucer, the branchial sense-organs, and the beginning of the lateral line. My observations on this region in *Squalus* agree with those of Mitrophanow.

In looking over the literature on the sense-organs one cannot fail to be struck by the remarkable uniformity of the sense-cells in the different kinds of sense-organs. They are easily reducible to one type, and this, of course, favors the view that they have been derived from a common form.

I. THE LATERAL EYES.

Squalus acanthias is an especially favorable form for observing the beginnings of the optic vesicles. They make their appearance in this animal at a very early stage, while the neural plate is broadly expanded, and before the neural folds have arched upwards in any part of the embryo. I should estimate their appearance in this animal to be as early as in any other animal in which they have been described. Previous to 1889 it had been current opinion that the optic vesicles appeared very early only in the class of Mammals. Bischoff, Kölliker, His, Van Beneden, Heape, and others had recorded their early appearance in that group; but it was regarded as a precocious development for some reason confined to mammalian forms.

More careful observations on the earlier stages have shown that the optic vesicles customarily arise in different animals much earlier than was supposed. In 1889 Whitman called attention to the very early appearance of the optic vesicles in *Necturus*: "Its basis being discernible as a circular area — after treatment with osmic acid, followed by Merkel's fluid — long before the closure of the neural folds of the brain." Dural noted the early appearance in birds. In 1893 Eycleshymer gave notes upon their appearance and structure in *Necturus* and other Amphibia. His most noteworthy observations are upon *Rana palustris*, a form hitherto unstudied, in which he finds a remarkably early development of the primary optic vesicles. They are very evident from surface study in this form, on account of the distribution of pigment granules in them, just after the neural ridges have begun to form. Cross-sections in these early stages show a considerable amount of histological differentiation from the surrounding cells.

The early differentiation of the optic vesicles had not been recorded in any elasmobranch form until the publication of my preliminary papers in 1893 and 1894, where the optic vesicles are described and their serial relation to other structures on the cephalic plate is shown. Since then I have had opportunity to confirm my observations on *Squalus*, and to extend them to some other forms; for instance, in *Diemyctylus* and

Amblystoma the optic vesicles are well developed in very early stages. In *Torpedo ocellata* they show fairly well, and in the chick they may be readily made out while the groove is widely open.

In *Squalus acanthias* the optic vesicles are the first rudiments of sense-organs to appear. Counting out the gastricular cavity (and the neural segments) they are the first organs of any kind to be established. Strangely enough, their early history in these animals has been entirely overlooked. Balfour's statement that "The eye does not present in its early development any very especial features of interest," seems to have withdrawn attention from that organ in the elasmobranch fishes.

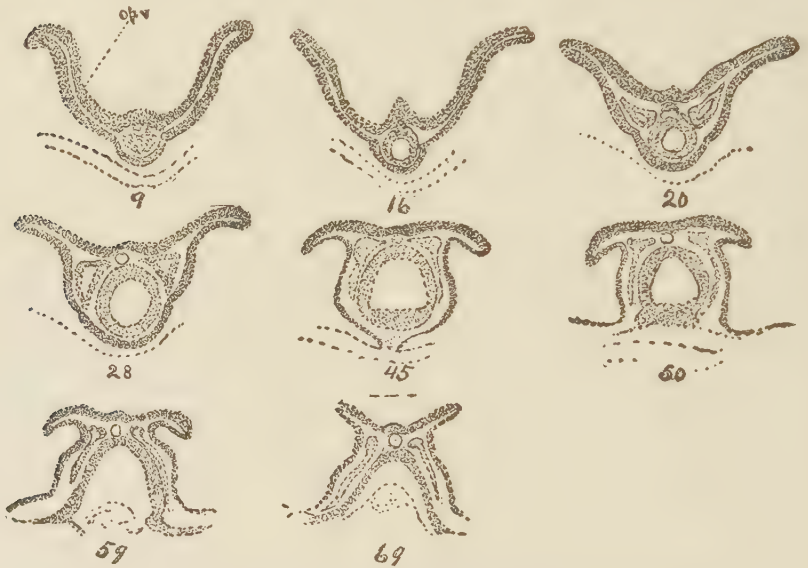
In order to fix clearly the time when the optic vesicles first appear in *Squalus*, we must glance over the early steps in the formation of the head-plate. Pl. XXVI, Figs. 1, 2, 3, represent three stages that immediately precede the formation of the eyes. In Fig. 1 the embryo is well established; it is a stage slightly older than Balfour's stage *B*. The front part of the embryo is not broader than the rest, and the curve of the anterior margin is uniform and unbroken. In Fig. 2 we note two changes, *viz.*, that the front end is broader than the part just behind it, which is apparently constricted, and the anterior margin is drawn out into a rounded median cusp. The head-end continues to expand laterally (Figs. 3 and 4) until we may (with some appropriateness) speak of it distinctively as a head-plate. In the meantime the trunk region has grown longer, and is relatively narrower, so that the entire axial embryo has a characteristic appearance of a narrow body terminated by a rounded plate-like head. When this stage has been reached the first satisfactory traces of the eyes become visible; when first formed they present the appearance that might be produced by pressing down lightly upon a plastic surface with two rounded dies. They are circular areas slightly concave upwards, and occupy a position far forwards on the cephalic plate. The early stage of these vesicles may easily escape observation, as they become evident only when the light strikes them properly, and when they are in a favorable position for the observer.

Fig. 5 is engraved from a photograph in which the optic vesicles show very well. The specimen from which the photograph was made showed about six mesoblastic somites, and was $2\frac{1}{10}$ mm. in length. The circular areas may be made out satisfactorily in slightly earlier stages, in which only three mesoblastic somites are differentiated, and in which the neural folds of both head and trunk are not only broadly expanded, but are even ventrally curved. The circular depressions are at first separated from one another by a raised welt. This has already been spoken of in Part I as a tongue-like process extending from the median-anterior tip backwards to two-thirds the length of the cephalic plate. It continues to be a prominent feature of the cephalic plate for some time. I can offer no suggestion as to its significance, outside of the obvious suspicion that it may represent a proboscis of some kind, or that it may be related to the large notochord of this region. On this point I have no conjectures to make. The depression to form the infundibulum starts a little after the first appearance of the optic vesicles, and cuts the median process in two, so that the anterior tip is separated from the rest, and the depression for optic vesicles and infundibulum combined reaches across the median plane of the cephalic plate (Figs. 6, 7, 8, 9, *etc.*). The optic vesicles, however, do not reach to the lateral margins of the neural folds; the latter are much expanded beyond them.

It is evident that we cannot, in a strict sense, speak of such vesicles as "diverticula of the fore-brain." The "diverticula" are found before the fore-brain. The depressions once started grow deeper and press outward laterally; when fully formed they are cupped, almost rounded in outline, concave from within, and they form rounded elevations where they come in contact with the outer layer of epiblast. Figs. 6, 7, 8, 9, 10, show very well the appearance presented by these primary optic vesicles when viewed from above. Fig. 7 is magnified higher than the others, and is, therefore, larger, but the others are all uniformly enlarged. These figures all show the interior of the optic pits. They represent a range of stages only slightly older than that shown in Fig. 5. In all of them the depression in which the optic vesicle lies extended across the median

plane, and in its median portion, the depression develops into the infundibulum.

The lateral depressions, which form the optic vesicle, sink downwards more rapidly than the median part of the depression, and as a result there is left a ridge or keel separating the optic vesicle and connecting the anterior-median tip of the head-plate with the central tongue-like process. Miss Platt, in her studies on the head of this animal, says: "It may be here

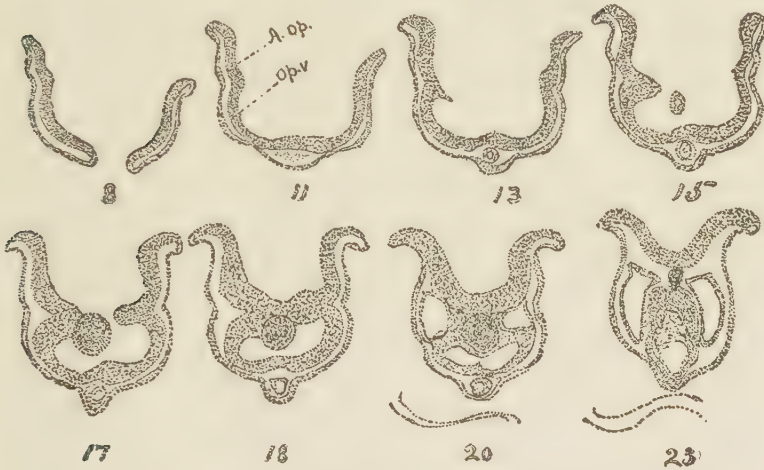


Cut 8. — Eight transverse sections of the embryo shown in Fig. 8, Pl. XXVI. $\times$ about 30 diameters. The numbers below the sections refer to their position in the series.

noticed that the downward growth in the floor of the brain, which gives rise to the infundibulum, is primitively bilateral, and not median," and I think, therefore, that she saw these circular optic pits in an early stage, but interpreted them as lateral pockets of the infundibulum. There might be reason to doubt whether they are the optic vesicles, if they could not be traced with perfect continuity into the eyes, and if there were not evidence of histological differentiation in their areas.

By the time the neural folds of the head begin to rise the optic vesicles are very prominent, and may be observed from the outside and from within. Fig. 13 shows an embryo viewed

obliquely from the left side, and the optic vesicle of that side is seen from the external surface, where it presents the appearance of a rounded eminence. On the opposite side the optic vesicle is seen from within. Cut 9 shows transverse sections of the head of the same embryo. Figs. 16 and 17 show embryos in which the optic vesicle (*op. v*) shows from without, and also from within (*op. v*). In Figs. 19 and 22 the optic vesicle shows clearly from the outside. Transverse sections of the

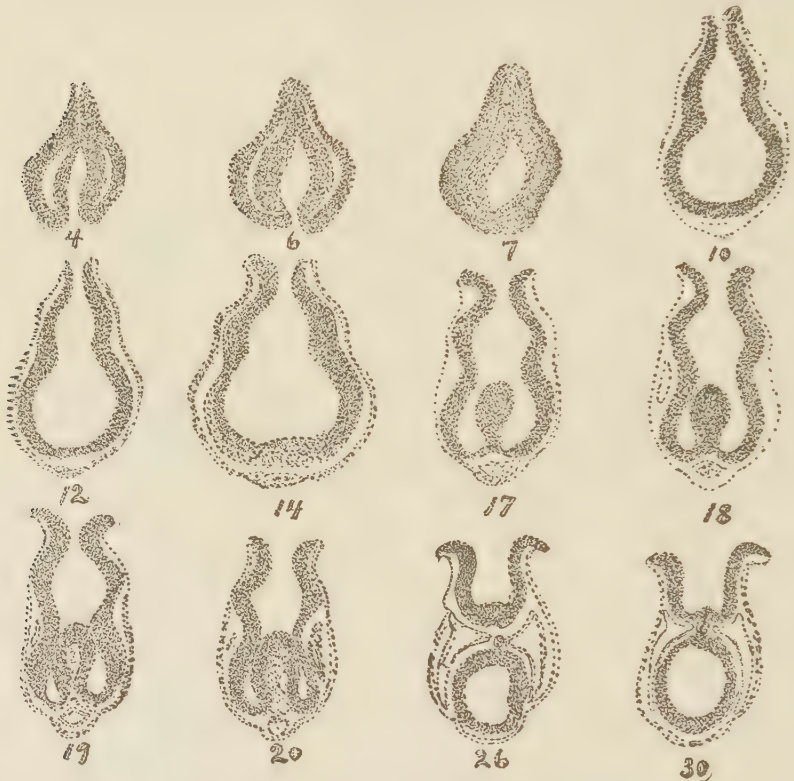


Cut 9.— Eight transverse sections of the embryo shown in Pl. XXVI, Fig. 13. $\times$ about 30 diameters. The accessory optic vesicles (*A. op.*) have been formed.

embryo photographed in Fig. 23, after partial closure of the neural groove, are illustrated in cut 10.

These optic vesicles are so well developed at an early period in *Squalus*, that it seemed to me probable that similar early formed vesicles might exist in *Torpedo ocellata*, and have been overlooked by the Zieglers, who have studied the early stages of that form in 1892; accordingly I procured embryos of *Torpedo ocellata* from the Zoölogical Station at Naples, and studied the head region with some care. The first thing noted was that the embryos of *Torpedo ocellata* are not nearly as favorable objects for observations as those of *Squalus*. They are smaller and the beginnings of sense-organs, branchiæ, and other structures about the head are by no means so clear as

they are in *Squalus*. As far as I am able to discern, there is no very distinct differentiation of the optic vesicles, in the early stages of this form; nevertheless, they are present at the stage designated *C*, by the Zieglers, and perhaps a little earlier. In studying the actual specimens, I had occasion to note that



Cut 10.—Twelve transverse sections of the embryo shown in Pl. XXVI, Fig. 23. $\times$ about 30 diameters. The numbers below the sections refer to their positions in the series. In Section 14 the optic vesicles show below and the depressions for the mid-brain vesicles above.

the Zieglers' models are admirable representations of the embryos of the form they are intended to represent. The optic vesicles are much fainter than they are in *Squalus*, but nevertheless are similar to them. I have studied them in *Torpedo* both in surface views and in sections. One noteworthy fact is that they have been sectioned and actually figured by Ziegler in his Pl. IV, Fig. 19¹. It will be noted in this figure, that the brain-walls bulge outwards on either side and these depressions

mark the interior of the optic vesicles. Pl. XXIX, Fig. 65, is a drawing of one of my sections of *Torpedo* slightly younger than the one of Ziegler's just referred to. The optic vesicles are indicated at *op*.

The external appearance of the optic vesicles in later stages is shown in Pl. XXVII, Figs. 34, 35, 36, 37. When the stage represented in Fig. 35 has been reached the lens shows externally; it is formed in the usual vertebrate way. The choroid fissure shows in Figs. 36 and 37. My studies have not been made to include the later stages of development of the optic vesicle, but are confined to its earliest differentiation.

Sections of the earliest-formed circular areas, show something in the direction of histological differentiation. Mitoses are more frequent and more of the cells are elongated and pear-shaped than in the other parts of the cephalic plate. The differentiation is by no means as marked as in the *Rana palustris* figured by Eycleshymer — nevertheless indications of change are not wholly lacking. My sections are too thick (10μ to 15μ) for satisfactory histological study, but one can see in them that the middle of the walls of the optic cups are areas of differentiation. The differentiation does not progress far until later, but the frequency of dividing cells, and their change of form continue, in these early stages, to be marks for distinguishing visual epithelium from that of the surrounding brain-walls. The dividing cells are neuroblasts, and these are known to be points from which the nerve-fibres spring. Their presence in any considerable number would, therefore, indicate a differentiation in the direction of increase of sensibility.

The eyes are the highest developed of the sense-organs, and in that particular stand at the head of the series. But, do the eyes belong to the same series with the other sense-organs, or do they occupy a position by themselves, can they be homologized with the rest? This is a puzzling question, over which there has been much controversy, and upon which there is still much difference of opinion among anatomists. The stock objection to their being classed with the other sense-organs, is this:—they have apparently been derived from a different basis, and their nerves are developed in a different way. The

eyes are formed out of neural-plate material as diverticula of the fore-brain, while the other sense-organs arise outside the neural plate ; they have an independent epiblast origin.

It is questionable, whether any particular stress should be put upon that argument, as the neural plate is at best only a part of the modified epiblast ; moreover, the suggestion that the neural plate is, undoubtedly, very much widened and expanded from its ancestral condition, and that certain sensory area, originally lying outside, may have been included in it, does much to offset that objection. The neural plate has been a prodigiously long time in forming, and the eyes have been brought into closer relationship with it. The plate is broadest in front, and the eyes are the most anterior sense-organs, and have become included in this expansion. While there is not sufficient data to give a wholly satisfactory answer to the question propounded, the assumption that the eyes are closely related to all the others is not without foundation, and we are now in the attitude of awaiting further facts.

II. ACCESSORY OPTIC VESICLES.

The neural plate, while still in very young stages, and while the neural grooves are widely open, becomes the seat of some accessory differentiations that resemble the optic vesicles. So far as I know no observations upon these structures have been recorded except those in my preliminary account in the JOURNAL OF MORPHOLOGY. I quote from that paper : But, more interesting than the fact of their (the optic vesicles') very early appearance in Elasmobranchs, is their apparent relationship to other depressions that are formed upon the cephalic plate, behind the already established optic vesicles. The new involutions referred to, make their appearance upon the cephalic plate just back of the optic vesicle. Two of them (Fig. 13, *ac. v* and Fig. 17, *ac. v*¹, *ac. v*²) take precedence of all others in development, and are so distinctly formed as to afford a good basis for comparison with the optic vesicles. They are circular depressions formed in front of the latter, and they produce upon the exterior corresponding rounded elevations.

The optic vesicles are formed first, and when, at a very little later stage, the others arise behind them, it appears as if the process of eye-formation were repeating itself serially.

These circular pockets not only arise in a similar way, but structurally resemble the optic vesicles. In cross and longitudinal sections the cells of the sunken patches are similar to those in the eye pockets. They may be designated *accessory optic vesicles*.

The assumption that they are primitively visual in character may be going too far, but the basis upon which it rests will be shown directly. The least that can be said of them (from their structure, the place and manner of their appearance), I think, is that they are segmental sensory patches.

These structures begin to appear soon after the eye vesicles. There are several pairs of them stretching in rows back of the eyes, as stated above, the two anterior pair are the most prominent. They gradually grow fainter; the anterior one is the best differentiated; the second one is not so clear, and the succeeding ones grow fainter as we pass backwards. I have counted as many as four pairs in surface study; but sections show that the series is more extensive and contains not less than eight pairs of these circular pits. The two anterior ones are well shown in Figs. 9 and 10. These are the earliest surface indications. In Fig. 17, which is slightly more advanced, the accessory structures are clearly developed; the figure shows four pairs behind the eyes. In longitudinal sections of the same specimen, Pl. XXIX, Figs. 76-87, eight pairs may be counted. The form of the depression, as seen at first from the surface view, is ovate; they soon become circular patches, and form themselves into concave, shallow cups. I have seen them in many specimens.

It will be noted that they make their first appearance while the neural plate is broadly expanded, and spring into prominence while the neural folds are growing upwards. It is during the rise of the neural folds that similar, but fainter, vesicles can be made out, in surface study, behind the two anterior pairs.

Pl. XXX, Figs. 88 to 112 show twenty-five transverse sections of an embryo very slightly older than that represented in

Pl. XXVI, Fig. 16. The sections 88 to 100 lie in the region of the cephalic plate. The following sections, 105 to 112, lie in the neck and trunk region. These sections are remarkable in bringing to light serial depressions along the walls of the neural folds. They show that the serial cup-like differentiations extend back of the cephalic plate. I have not been able to determine the number of serial differentiations of this character in the embryo, but it is clear that there are several pairs behind the cephalic plate upon which I have noted, in surface study, four pairs in addition to the primary optic vesicles.

My sections are not favorable for a critical study of the histological conditions, but it is clear, from them, that a differentiation starts in the anterior patches similar to that mentioned above, for the true eye vesicles. There is a greater frequency of dividing cells and many of the cells become elongated and pear-shaped.

Comparisons of the two anterior pairs with the eye vesicles give the following points of resemblance. They are formed in precisely the same way, and present the same general appearance; viewed from within they are all similar concave cups; they produce corresponding elevations on the outer surface, and, if observed from the outside, they form a series of rounded knobs serially arranged, the eye being the anterior terminations of the row. The marked differentiation in the eye takes place in later stages. There are, however, the histological features spoken of above that serve to distinguish it in early stages, and the accessory vesicle shows the same characteristics.

These resemblances, coupled with the existence of serial eyes in some of the Invertebrates, has led to the assumption that in Vertebrates these rudiments represent accessory optic vesicles. The fate of the anterior pair would also favor this view; they pass into the pineal sense-organ, a structure whose visual character is acknowledged.

If the view expressed above is true, we have a multiple-eyed condition in the embryos of these animals. This is common enough in Invertebrates, but has not been previously noticed in Vertebrates.

These accessory structures are present for a brief time only. That region of the head, behind the optic vesicles and in front of the ears, becomes the seat of great modifications, and, as the neural groove closes, the structures described give way to later formed ones. They are, therefore, not only embryonic structures, but are also transitory. It is a truism in developmental history that the more primitive characteristics appear first, and the secondary modifications come in later. The structures described are among the most primitive that have been preserved in this very ancient group of Vertebrates, and should be of significance in indicating the ancestral relations of the eye. I have spoken of the disappearance of the organs, but I have been able to trace the anterior pair further along, an account of which will be given in the next section on The Pineal Sense-Organs.

I have also traced out similar rudimentary organs in the embryo chick, at about the conventional 24-hour stage; there is a series of seven vesicles behind the optic vesicles. These structures are rudimentary, and very quickly fade away.

All this acquires new interest when taken in connection with the researches of Whitman on the eyes of leeches. As indicated above, he showed that the eyes of the leeches are derived from segmental sensory papillæ. It now appears that we have traces of a somewhat similar line of segmental sensory organs in Vertebrates. It is essential to say *traces*, for these structures are transitory, and do not rise to a high grade of differentiation, except in the case of the pineal sense-organs. They may never, in the vertebrate group, have been raised to the rank of eyes, but it is certain that in Annelids similar segmental sensory patches have been so raised. It is, however, reasonable to suppose that members of this series have been functional, even in Vertebrates, when we recollect the highly developed condition of the pineal eye in some forms.

There is really very little direct evidence to support the proposition that the Vertebrates are derived from multiple-eyed ancestors, although it is a conception that has been in the minds of morphologists for some time. Besides the indications of such a condition afforded by the facts of this paper there

has recently come more evidence bearing in the same direction. This consists in the discovery of multiple pineal eyes, in several distinct forms, and, in one case, the existence of three distinct nerves to the pineal organs (see section on The Pineal Sense-Organ).

Whitman, in his paper just cited, says: "Although the evidence appears to me conclusive that the eyes and the segmental papillæ were, originally, morphological as well as physiological equivalents, it does not, of course, follow necessarily that both organs now have the same functional significance. The original papillæ may have represented sense-organs of a more or less indifferent order, among which, in the course of the historical development of the leech, a division of labor was introduced, a few at the anterior end becoming specialized as light-perceiving organs, the rest either remaining in their early indifferent condition or becoming specialized in some other direction.

"The discovery that these papillæ are sense-organs might lead us to speculate on affinities of a distant and somewhat uncertain nature, such as are supposed by the writer, in common with many others, to exist between annelid worms and Vertebrates. At all events, the existence of such organs in the leech furnishes a broader basis for the discussion of the question whether the Vertebrates and Annelids have been derived from a common form possessing metameric sense-organs, as was first argued by Dr. Eisig of the Naples Station. Assuming that the sense-organs of the Vertebrate and the segmental papillæ of the leech may be traced to a common origin in some remote ancestral form, it does not follow that they should now present close structural resemblances. It is far more important to show that they possess certain general features in common. The most important of their common features is undoubtedly their metameric origin. The nerve-supply forms another feature of fundamental importance, in which, according to the interesting observations of Mr. Beard, on the segmental sense-organs of the lateral line (*Zool. Anz.*, VII, Nos. 161 and 162) of the Vertebrate there is essential agreement. The developmental history of these

lateral organs in the fish, where they make their first appearance as *segmental papillæ* in the strictest sense of these words, cannot be explained on a more satisfactory hypothesis."

III. THE PINEAL SENSE-ORGANS.

1. *Growth of Knowledge regarding the Pineal Sense-Organs.*

The pineal outgrowth has attracted much attention since the discovery, in 1886, of its eye-like structure. Since then it has been accepted by morphologists as a rudimentary sense-organ. The earlier literature bearing on the subject has been thoroughly reported on by Spencer ('86) and Francotte ('89). Several recently published papers taken together put the subject in a new light, and, as Ritter ('94) has said, "At no time since the epiphysis and pineal eye have been the topic of investigation has it been a more interesting or a more inviting one than it is just now."

The growth of our knowledge regarding this remarkable sense-organ, scattered through the various publications of Spencer, Béranek, Francotte, Strahl and Martin, Leydig, and others, may be reduced to the following summary :

First came the demonstration of its eye-like structure in Amphibia, by De Graaf, and in Lacertilia by Spencer. De Graaf's publication is much briefer, and was published only a few months before Spencer's extensive study. Both authors investigated the structure in adult forms. Spencer studied its condition in twenty-eight different species of Lacertilia, and demonstrated very clearly that even in the adult forms of these animals, this organ has an eye-like structure, with a lens, a pigmented retinal layer, and a nerve. It seems doubtful, in the light of recent work, whether the fibrous strand connecting the eye-capsule with the epiphysial stalk, and designated the nerve by Spencer, is really nerve or not.

Following the study of the adult structure came investigations of the embryonic conditions. The first studies dealing with the embryonic development of this organ, and with its

minute structure in the early stages, were those of Strahl and Martin. Along this same line were the studies of Béraneck, Francotte, Leydig, and others. By these investigators the organ was traced backwards to a certain point in its history, that is, to the time when it springs from the roof of the thalamencephalon as a tubular outgrowth, like the tip of a finger of a glove. To this period also belongs the discovery of a nerve of transitory existence. The investigations were confined almost entirely to two groups of animals, Amphibia and Reptilia. In the embryos of these animals many points of minute structure were worked out, such as the distribution of the nerve within the eye-like capsule, the division of the retinal part into distinct layers, the fact that the pineal eye is higher developed in the embryonic periods than later, etc.

The next step was the discovery that there is more than one epiphysial outgrowth. Selenka, Leydig, Eycleshymer, Hill, successively called attention to the existence of two distinct outgrowths, or vesicles, from the roof of the thalamencephalon in Anguis, Amblystoma, and Teleosts.

Hill's studies on these two vesicles are the most complete. He has shown the existence in Teleosts and *Amia* of two vesicles, anterior and posterior, and has traced them through the stages of development. The posterior develops into the epiphysis and the anterior degenerates. The distal portion of the former is histologically very complex, and receives a nerve from the posterior commissure. Hill made out the distribution of nerve-fibres in this part of the epiphysis. He traced quite clearly the history of the vesicles.

Two vesicles, an upper and a lower, have long been known to exist in *Petromyzon*, in both embryos and adults. It is much to be regretted that we do not know their embryonic history, for much depends on this. The most recent paper on the epiphysial organs in *Petromyzon*—that of Studnička—does not clear up the question of the origin of the two vesicles. Histological material was lacking in just the stage required. The two outgrowths in embryos outside the Cyclostomes have been designated epiphysis, for the hinder, and pineal eye for the anterior. Studnička designates them pineal and parapineal,

respectively, in the Cyclostomes. As I shall show later, the so-called paraphysis is a different structure.

Béraneck showed that the pineal eye and epiphysis have been confused, and, on account of the existence of the nerve from outside sources, he argued for the complete independence of the pineal eye.

Klinckowström has placed himself in opposition to this opinion, showing the eye vesicle is formed as an outgrowth from the epiphysis.

Next to the discovery of two or more epiphysial outgrowths may be mentioned the existence of accessory pineal eyes. The earliest publication containing an account of such structures is that of Duval and Kalt, in 1889. In a brief note (*Des Yeux Pinéaux Multiples chez l'Orvet*) these authors record the finding of two or three accessory pineal vesicles in *Anguis*. They do not say whether adult or embryonic forms were studied.

Carrière ('89), in the same year, noted the occurrence in embryos of *Anguis* of a very rudimentary accessory pineal vesicle.

Leydig ('90) observed these structures independently in embryos of the same animals. He showed that there is much variability, both as to the presence and the histological structure of these accessory capsules. In some individuals they are lacking, and in others of the same age present and well developed. He records the occurrence, in some cases, of two accessory organs, a larger and a smaller one. The larger one resembles the pineal eye in structure and arrangement of pigment. The smaller one is very rudimentary. Leydig compared them to the ocelli of insects. It is clear from his account that variability and inconstancy are characteristics of these accessory organs.

Prénant, in the latter part of 1893, again records the occurrence of one accessory pineal eye in the embryos of *Anguis*. He directs attention to the fact that up to that time they had been discovered only in a single species, *viz.*, *Anguis fragilis*, and further, that they were all in embryos, and not in adults. Ritter ('94) has recently recorded the occurrence of such an accessory organ in the adult *Phrynosoma*.

The above observations, taken together, give sufficient positive data to establish the fact that there appears, from time to time, in some animals the vestiges of accessory pineal organs, and that they are variable and inconstant in their occurrence.

The most recent advances consist in carrying the history of these organs backwards, and showing them to be connected with patches of sensory epithelium that arise on the cephalic plate in very young stages, and in the discovery, in *Iguana*, of three distinct nerves connected with the epiphysial outgrowths. The former work was done by the present writer, and the latter by Klinckowström.

As indicated in the preceding section, there are several pairs of cups on the cephalic plate back of the optic vesicle, formed while the neural groove is widely open. I have designated them accessory optic vesicles. In the process of closure of the neural groove the anterior pair are brought together, and form part of the thalamencephalon, from the roof of which the pineal outgrowth is derived. This process will be described more in detail.

Klinckowström ('93) has recently shown in *Iguana* the presence of two nerves connected with the anterior outgrowth or pineal eye, and sometimes a third connected with the posterior outgrowth or epiphysis.

These observations suggest new interpretations; still it is not an auspicious moment to indulge in speculation. It is clearly evident that we need more data regarding these organs and their relationships. But the trend of these discoveries is to strengthen the suggestion already made in this paper, that, primitively, the Vertebrates were multiple-eyed. The presence of accessory pineal eyes, the discovery of serial sensory areas on the cephalic plate from which epiphysial outgrowths arise, and the presence of two or three pineal nerves, are all consistent with this interpretation. So far as the evidence goes, there is more than one epiphysial outgrowth, and therefore I have headed this section, *The Pineal Sense-Organ*s. In what way it will be necessary to qualify this proposition will depend on subsequent discoveries.

The results of all the embryological studies on the epiphysial outgrowth between 1887 and 1893 were to settle on one point of common agreement regarding its origin. Each investigator successively found it to arise as a tubular outgrowth from the roof of the thalamencephalon comparatively late in ontogeny, after the parts of the brain are established. No earlier trace of it had been found; but there was a previous undiscovered history, and it is now timely to make the inquiry, What is the very beginning of the pineal sense-organs?

2. *Remote Origin of the Pineal Outgrowth.*

As already indicated, there are upon the cephalic plate accessory eye vesicles which have made their appearance while the neural groove is widely open; and in tracing their fate I shall attempt to fill up that gap in the history of the pineal outgrowth from the time these cup-like structures appear upon the cephalic plate to the time the tubular outgrowth begins from the thalamencephalon.

As the walls of the neural groove grow upwards these cup-like structures are carried up with them; and there comes a time (Figs. 13, 31) when they may be seen from the outside as rounded eminences, and from the inside as concave cups. When the edges of the neural groove meet in the middle line the cups are approximated, and come to form part of the thalamencephalon.

While these changes have been going on, further differentiations have been taking place in the walls of the brain. The bulging of the walls to form the mid-brain vesicle has come on insidiously, and has taken a position behind the vesicles of the paired eyes, in apparently the same position previously occupied by the accessory vesicles. These transformations are confusing, as the walls of the mid-brain resemble the accessory optic vesicles grown larger. In an earlier published paper I made the mistake of identifying the mid-brain with the accessory optic vesicles; but it was merely an error of identification, and did not affect the main contention of that article.

In working out the details of the formation of pineal outgrowth in *Squalus*, it soon becomes apparent that the surface

contours of the head are considerably altered by the distribution of mesoblastic cells lying between the external layer of epiblast and the brain-walls. The mesoblast forms a pad of varying thickness in close contact with the brain-walls; the depressions are filled, and in some places thick patches of mesoblast give rise to external markings that render the surface appearances untrustworthy. There is, for instance, a deep pad of mesoblast filling up a depression at the sides of the cerebellum, and also extending forwards on to the mid-brain; at the external surface the pad assumes a circular form, and it is that pad of mesoblast which is seen in Fig. 32. The true mid-brain vesicle in that figure is the eminence marked *mb*. The accessory optic vesicles are present, but are not well marked externally. They are in front of the protuberance marked *mb*.

In order to get a view of the brain-walls I have found it necessary to remove the mesoblast and its coverings of epiblast, and thus to completely expose the brain-walls. The brain thus laid bare, enables one to see with complete satisfaction its different parts and their relation to one another. A very interesting relation comes to light through the dissections. Somewhere between the stage with an open neural groove (Fig. 31) and the completely closed groove (Fig. 33) the mid-brain vesicle insidiously takes the former position of the accessory optic vesicle while the latter is carried forward by the process of cranial flexure. During the formation of the mid-brain vesicle the two structures become incorporated into one faintly bilobed protuberance (Pl. XXVIII, Figs. 38 and 39). The anterior part is the accessory optic vesicle, and the posterior one the mid-brain. The separation soon becomes complete, and by the stage represented in Fig. 40 the two are completely separated; but owing to the arrangement of the mesoderm the accessory optic vesicle is rendered indistinguishable from the outside. When the mesoderm is entirely removed it may be seen. All this takes place in very brief intervals of time, and a complete series of embryos representing the different phases of the closure of the neural groove is required to see it all. I must say also that the changes are so perplexing

that there exists in my mind some doubt as to the adequacy of the above account. It may have to be modified, but I have given the facts as they now appear to me.

Figs. 38-54 show a series of embryos that have been partly dissected in the way just indicated. They show characteristic changes in the brain-walls as seen from the exterior.

In Fig. 38 the walls of the neural groove have not yet met in any part of their course, and the thalamencephalon cannot be distinguished from the rest of the brain. The first pair of accessory vesicles are visible; they soon become incorporated in the walls of the thalamencephalon. I have not been able to determine whether it is the epithelium of the first pair of accessory vesicles only, or whether epithelium from the second pair is also included. Either the epithelium of the two pairs is incorporated into the walls of the thalamencephalon by being carried together, or the epithelium of the second pair fades into the surrounding substance of the brain-wall, which almost immediately grows into the vesicle of the mid-brain. Although I am doubtful as to whether the second pair of accessory vesicles pass into the inter-brain, I am sure that the epithelium of the anterior pair is so included.

Fig. 39 shows the first accessory optic vesicle and the mid-brain vesicle forming a common protuberance, but they are very quickly separated, and the anterior vesicle goes into the thalamencephalon.

In Fig. 40, which is somewhat older, this has occurred. The lateral walls of the thalamencephalon now consist of two shallow cups, approximated so that the structure looks lenticular when viewed from above. As a usual thing, the thalamencephalon is not evident at this stage before the removal of the overlying tissues. Fig. 41 shows, however, a specimen in which it could be seen before any dissection. Compare this with Fig. 32, which is the more usual appearance of an embryo of this age.

Fig. 42 is a dissection of the embryo shown in Fig. 41. The mid-brain, which, from external view, appears like a single rounded eminence, is shown after exposure to be bilobed.

Figs. 43, 44, and 45, which are successively older, show an increase in the size of the thalamencephalon, and marked changes in the mid-brain.

In Figs. 46 and 47 a faint furrow has appeared in front that serves to mark the boundary between the thalamencephalon proper and the cerebral lobes. This furrow is clearly defined in Figs. 48 and 49, so that we have now the thalamencephalon distinctly marked off from the rest of the fore-brain.

In Fig. 48 the optic vesicle and all the mesoblast have been removed, and the view is taken from the right side. The brain-walls show very clearly. The thalamencephalon is now bounded anteriorly and posteriorly by furrows. The two furrows run nearly parallel to one another, and they serve to mark off the inter-brain very distinctly.

The roof of the thalamencephalon is at this stage raised into two rounded eminences of nearly equal dimensions, an anterior and a posterior one. The posterior is, from the beginning, somewhat in advance of the other. It becomes the pineal outgrowth, while the posterior eminence goes on developing. The anterior one becomes greatly reduced by compression from the rapidly growing adjacent brain-walls. It very soon loses its rounded character, and becomes pressed into a semicircular fold lying in front of the epiphysis. It is, I think, equivalent to the Zirbelpolster of German authors. If longitudinal sections be made at the stage represented in Fig. 48, the two elevations show as in Minot's Fig. 319, p. 572, of his *Human Embryology*. In that figure they look like two equal vesicles springing from a single elevation of the brain roof, and opening by a common wide passage into the brain vesicle. No especial significance, I think, is to be attached to the fact that there are at first two equal embossments from the roof of the thalamencephalon. The anterior one is converted into a fold of the roof of the 'tween-brain that has long been recognized in different animals. It is designated Zirbelpolster by Burckhardt. It does not in any sense correspond to the anterior epiphysial vesicle discovered by Hill in Teleosts, but rather to the fold of 'tween-brain that lies in front of both epiphyses. Studnička has, however, marked the fold in diagrammatic figures of Tele-

osts, the anterior vesicle of Hill, but this identification is not right.

We have in this specimen the first appearance of the tubular outgrowth, which, according to previous authors, marks the beginning of the epiphysis. It is somewhat important to fix the stage at which this outgrowth begins. Its very first appearance as an elevation of the roof of the thalamencephalon is in the stage represented in Fig. 36 after the saucer-stage of the ear is past.

In Fig. 49 the posterior protuberance of the roof of the thalamencephalon is assuming a tubular form. It becomes the epiphysis. The surface of the mid-brain is now considerably changed. Two furrows have made their appearance, which divide the lateral walls into three nearly equal lobes. These do not show at all from the exterior before the removal of the epidermis and the mesoblast.

The gradual reduction of the thalamencephalon by compression between the central hemispheres and the mid-brain is shown consecutively in Figs. 53, 54, 55, 57, and 60. In Fig. 54, and later, the epiphysis appears pear-shaped from the side, but from in front the enlargement on the distal end is seen to be broader than thick. It is borne upon a hollow stalk.

Fig. 57 represents the brain of an embryo considerably older than in Fig. 56. The thalamencephalon is now very much compressed. The anterior half of it, which originally had a rounded protuberance growing from its roof, is now reduced to a small semicircular fold in front of the epiphysis.

Fig. 59 is a view upon the epiphysis of the same embryo from directly in front. It was obtained by removing the cerebral lobes, which lie in front of, and partly hide the epiphysis. The epiphysis is seen to be composed of a stalk and an enlarged distal extremity; leading from the stalk on either side are the epiphysial peduncles. In this figure we are looking through the ventricle of the thalamencephalon into that of the mid-brain. On the inside of the lateral walls of the opening are two enlargements, the beginnings of the thalami optici. These contain the ganglia hebenulæ.

In this specimen the paraphysis had made its appearance as an outgrowth from the cerebrum. It is relatively late in arising, and is entirely distinct from any outgrowth from the thalamencephalon. The lateral walls of the mid-brain are no longer lobed as in earlier stages. Fig. 58 is the same brain viewed from above; we are looking directly into the cavity of the fourth ventricle.

Fig. 60 shows the brain of a still older embryo. It is the largest embryo of *Squalus* in my collection, measuring 35 mm. in length. The cerebral lobes are divided into right and left halves by a shallow furrow. From the back of the cerebrum rises the paraphysis (*p*); just behind this is seen the semi-circular fold, belonging to the 'tween-brain, and behind that is the epiphysis, rising above and resting upon the anterior wall of the mid-brain. Fig. 61 shows a front view of the cerebral lobes after being removed from the rest of the brain; the paraphysis is also brought into view. Fig. 62 is the same brain with the cerebral lobes removed, and placed so as to give a full-face view on the epiphysis; the stalk is considerably longer than in Fig. 59.

I have not had material to work out the subsequent history of the epiphysis. In specimens about six inches long it is situated in the wall of the cranium, directly over the cerebral lobes, and is connected to the roof of the 'tween-brain by a long thread-like stalk. The enlarged distal end is about the same size as in Fig. 62. Cattie shows it in the adult with several capsules on the end of the stalk.

Sections of the stages just studied serve to confirm the observations made from the outside. They do not add very much to its features, speaking strictly morphologically. The surface views have been prepared in such a way that they may stand for reconstruction, but they are more accurate than any actual reconstruction can be, as they show the relations entirely undisturbed.

Figs. 119-124 show a series of sagittal sections covering the period of the first appearance of the epiphysis, the reduction of the anterior part of the thalamencephalon, and the beginning of the paraphysis and the choroid plexus.

Fig. 119 is a specimen of the same age as that shown in Fig. 48. It shows that the roof of the thalamencephalon is raised into two nearly equal protuberances.

Fig. 120 shows the beginning of the reduction of the anterior of these elevations, and the increased growth of the posterior one. In Fig. 121 the posterior elevation, or epiphysis, has become a tubular outgrowth, while the anterior one is reduced and depressed.

In Fig. 122 the tubular stalk of the epiphysis is considerably elongated, while that part of the roof of the thalamencephalon in front of it has become reduced to a narrow fold that has been carried ventrad. The paraphysis has now arisen from the roof of the prosencephalon. The beginning of the choroid plexus extends from the structure down into the ventricle of the fore-brain, and its posterior part is connected with the fold in front of the epiphysis. The anterior and posterior commissures are now distinguishable.

Figs. 123 and 124 exhibit substantially the same relations; the choroid plexus has considerably increased in extent, and is rapidly becoming much convoluted; the brain vesicles have become reduced. In Fig. 124 the distal end of the epiphysis is inserted into a cavity in the roof of the cranial wall, that is, the mesenchyme forms a cup over its rounded end.

3. *Comparisons between Epiphysial Outgrowths.*

The recent studies have done much towards establishing a basis for comparison between the epiphysial outgrowths in different groups of animals. Béraneck, Francotte, and others claim that the anterior epiphysial vesicle — commonly designated the pineal eye — is formed independently, having no generic connection with the posterior one, or epiphysis. Hill's observations on the Teleosts coincide with this view. Klinckowström, on the other hand, claims that the anterior one is formed at the expense of the posterior one, and substantiates his conclusions with figures of sections of Iguana, showing the two vesicles in union with one another. It is not difficult to conceive how the condition represented by Kline-

knowström might be brought about, and still not be inharmonious with the other observations. The two vesicles might, in earlier stages than he represents, have been formed independently, side by side, and thrown into communication by an upward growth of the brain roof involving them both. Or, indeed, there may be variations in the details of the formations of these two vesicles.

However this may be, the results of the different observers all go to show conclusively that there are two (not counting the paraphysis) distinct outgrowths from the roof of the thalamencephalon of *Petromyzon*, *Teleosts*, and *Lacertilia*. The fate of the anterior vesicle varies in the different groups, while the posterior one persists in all as the epiphysis. The anterior one may be formed as a rudimentary organ of transitory existence, as in *Teleosts*; or it may be developed into the pineal eye, in front of the epiphysis, as in *Lacertilia*.

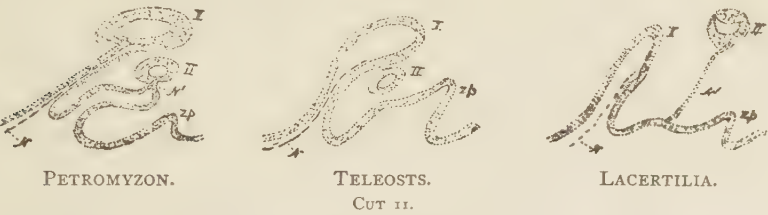
The sources of nerve-supply to the two vesicles have been made known in *Cyclostomes*, *Teleosts*, and *Lacertilia*, and these facts are important in establishing homologies. It has been shown (*Studnička*, *Gaskell*, *Klinckowström*) that the upper capsule of *Petromyzon* receives its nerves from the posterior commissure, and that the lower vesicle is innervated from the left ganglion hebenula. *Hill*, in embryos of *Teleosts*, has traced a nerve from the posterior commissure into the epiphysis and has studied its distribution in detail in that structure. The anterior vesicle of *Teleosts* disappears before it has formed any nerve connection with the brain.

Klinckowström ('93) has observed very interesting conditions in embryos of *Iguana*. In this animal he found in one case three nerves distributed to the parieto-pineal organs: one coming from behind and entering the epiphysis, and two coming from right and left ganglion hebenula, respectively, and entering the pineal eye. He found the right and left nerves in three different embryos of *Iguana* eighteen days old. In one case the nerve from the left ganglion hebenula was nearly the same size as that from the right, but in the other two individuals the left nerves were much reduced. It should be added that *Klinckowström* has found the nerve to the pineal

eye in this animal to come normally from the right ganglion hebenula, while in *Petromyzon* it comes from the ganglion of the left side. But, his discovery of the occasional presence of two nerves in *Iguana*, the left one being smaller, affords an interesting transition between the two. It also enables us to account for the marked asymmetry in the ganglia hebenulæ in *Petromyzon*, on the hypothesis that the right ganglion, in *Cyclostomes*, has, for some reason, ceased to have any nerve connection with the pineal organ, while the left ganglion has retained its connection.

As Béraneck has shown, a nerve leading to the pineal eye arises in front of the epiphysis, and has but a transitory existence.

Putting the facts together and basing relationships on the nerve-supply, and comparative study of the vesicles, we may express the probable homologies in the following diagrams and table :



- I. Upper vesicle in *Petromyzon*, epiphysis in *Teleost* and *Lacertilia*.
II. Lower vesicle in *Petromyzon*, Hill's anterior vesicle in *Teleosts*, pineal eye in *Lacertilia*.
N. Nerve to posterior commissure. N¹. Nerve from lower vesicle in *Petromyzon* to Ganglion hebenula, embryonic nerve of transitory existence in *Lacertilia*.
Z.P. "Zirbelpolster."

	PETROMYZON.	TELEOSTS.	LACERTILIA.
Posterior epiphysial vesicle of Hill. Pineal organ of Studnička. Epiphysis of others. } I. {	Is the superior vesicle. Nerve-supply from posterior commissure.	Is the epiphysis with nerve connecting it to posterior commissure.	Is the epiphysis nerve-supply from posterior commissure.
Anterior epiphysial vesicle of Hill. Parapineal organ of Studnička. } II. {	Is the inferior vesicle.	Is formed as in the two other groups, but aborts.	Becomes pineal eye supplied with a nerve which early degenerates.

If these comparisons are well taken, we have in the *Petromyzon*, the epiphysis in its highest state of development. Its distal end is enlarged into an eye-like capsule that is more differentiated than their inferior vesicle which represents the pineal eye. This is the reverse of what is true in most *Lacertilia*, and, on this account the upper capsule of *Petromyzon* has usually been compared with the pineal eye of *Reptilia* and *Amphibia*. But the fact that the superior capsule in *Petromyzon* receives its nerve-supply from the posterior commissure, corresponding in this particular with the epiphysis of other animals, has much more weight in determining its homologies than any purely structural resemblances. We are on a better foundation, therefore, in comparing the upper capsule of *Cyclostomes* to the epiphysis of other animals, than in taking the other position. It may be noted, in passing, that the superior capsule is in the same relative position as the epiphysis, and the inferior one corresponds in position with the pineal eye.

This view would make the epiphysis eye-like in character, and there are many facts that weigh in that direction. Hill's studies speak strongly for the visual nature of the epiphysis. He shows a high degree of differentiation in the nerve cells to which the nerve from the posterior commissure is distributed. There is also formed in *Iguana*, *Plica*, and *Phrynosoma* an eye-like enlargement at the distal extremity of the epiphysis.

Between the condition in *Petromyzon* and that in the *Lacertilia* many gradations have already been brought to light.

In *Teleosts*, the anterior vesicle arises, as in the other groups, but it is transitory, and soon degenerates. It would appear from a recent paper by Ritter, that the anterior vesicle in *Phrynosoma* is more persistent than in *Teleosts*, but does not reach the grade of differentiation exhibited in the *Lacertilia*. He gives a figure showing the rare occurrence of the anterior vesicle in an adult *Phrynosoma*, and although large this capsule is not so highly differentiated as the capsule behind it, but to compensate for this the distal end of the epiphysis is highly developed. In other forms of *Lacertilia*,

the anterior vesicle reaches a high grade of perfection, while the epiphysis is not well developed. In most cases, however, there is a deposit of pigment in the distal position of the epiphysis. The nerve distribution in that organ is known only in the Teleosts.

We might carry our comparisons further and inquire whether the structure present in Selachians is to be homologized with the epiphysis or the pineal eye of other forms. The nerve relations are not known in the Selachians and, therefore, we have not, as yet, a satisfactory basis for comparison. In its structure and persistent attachment to the brain roof by a stalk, the outgrowth in Selachians resembles the epiphysis and one would be inclined to say that only the epiphysis is represented in the Elasmobranchs, and that the pineal eye is lacking. Klinckowström has offered the ingenious suggestion that in Selachians and birds the second or anterior epiphysis is never fully separated from the posterior, and this is worth thinking about. There is no positive evidence to support it.

However the above question may be settled, it seems to me that the fundamental features of the morphology of the epiphysis are tolerably clear, and that future work on this subject will be mainly in the direction of amplification and discovery of details.

The invertebrate homologies of the pineal eye are not known. Spencer supposed it to be homologous with the median eye of Tunicates, but that relation is a strained one.

Various authors have compared the pineal outgrowths to the ocelli of Arthropods. Leydig compares them to the stemmata of Insects, and shows that there are many resemblances to support the comparison.

Patten has recently studied the development of the eyes of *Limulus*, and argues for a homology between the median eyes of these Arthropods and the pineal eye of Vertebrates. He shows the median eye of *Limulus* to arise by the fusion of paired eye-stalks, giving us a case of undoubted union of originally paired sense-organs. I have claimed a similar occurrence in the epiphysis of *Squalus*. But it seems to me that the

evidence is too circumstantial at present to bear the interpretation that we already know the invertebrate homologue of the vertebrate pineal organ.

4. *The Double Nature of the Epiphysis*

receives support from two sources. It is difficult to interpret Klinckowström's discovery of a double nerve in Iguana on any other hypothesis. That interpretation is also strengthened by the claim I have made of tracing the epiphysis in Selachians back to a pair of epithelial cups on the cephalic plate.

Hill ('94) regards the two vesicles he has discovered in Teleosts as having been primitively side by side ; and Ritter ('94) has recently suggested that the epiphysis and the parapineal organ of Studnička are right and left mates rather than independent eyes of double origin. It will be remembered, in this connection, that Klinckowström found three nerves in one individual of Iguana, two from the ganglia hebenulæ, and one from the posterior commissure ; that Leydig discovered two accessory pineal organs in Anguis ; Duval and Kalt found as many as three of these organs, and I have found several pairs of epithelial patches back of the eyes on the cephalic plate, and have traced one pair into the epiphysis. It seems to me that all this evidence is more favorable to the idea that the pineal sense-organs are multiple, and individually of paired origin, than to the idea expressed by Ritter.

5. *Paraphysis.*

Considerable confusion has arisen in identifying the paraphysis in different forms. As soon as it was made known that there are two outgrowths from the roof of the fore-brain, it was at once assumed that the most anterior one is the paraphysis, and the posterior one the epiphysis. But there is now evidence that there are, at times, more than two outgrowths. Hill has shown in *Amia* the presence at one and the same time of three tubular outgrowths from the roof of the fore-brain. Two of these come from the thalamencephalon, and the anterior one arises from the prosencephalon. He points out that

the latter is the paraphysis proper, and that in *Amia* we have to deal with two other outgrowths from the roof of the thalamencephalon. These he makes homologous with the two vesicles he has described in Teleosts. Hill concludes that in the latter group the paraphysis is lacking on account of the non-development of the choroid plexus. This identification is in harmony with the original description of the paraphysis. Francotte, who described it in 1887, and Selenka ('90), to whom the credit of its discovery is usually accorded, both state that it arises from the prosencephalon. Selenka says: "Wie das Zwischenhirn seine Epiphyse, so hat das Vorderhirn seine Paraphyse." Francotte suggested that it represents the beginning of the choroid plexus.

Minot ('92), p. 690, designates the anterior vesicle discovered by Hill ('91) in *Coregonus* the "paraphysis," but the structure in question arises from the roof of the thalamencephalon, and between it and the prosencephalon is a marked downward fold in the brain-wall, which in many other forms is present behind the paraphysis. It is more probable, as Hill suggests in his later paper ('94), that the paraphysis is lacking in the Teleosts.

It is to be understood that in some forms there are two epiphysial outgrowths from the thalamencephalon that are entirely independent of the paraphysis. When the latter structure is present in conjunction with the former two, as in *Amia*, there are then three separate outgrowths from the roof of the fore-brain.

As already indicated in this paper, the paraphysis is present in *Squalus* as an outgrowth from the prosencephalon, and is connected with the choroid plexus.

IV. THE BEGINNING OF THE AUDITORY ORGAN.

The formation of the auditory saucer is preceded by a thickening of the epidermis along the sides of the head in the auditory region. The thickening portion occupies a larger area than that used by the ear vesicle when it is first formed, and it is confluent with the epidermal thickening just above the

branchiae. As Mitrophanow has pointed out, this thickened patch of epithelium is composite in character; it embraces not only the epithelium for the formation of the auditory plate, but that to form the so-called branchial sense-organs, and also the beginning of the lateral line. I had noted this relationship in *Squalus* before seeing Mitrophanow's paper, and I find no comparison that my results correspond — so far as the earlier stages are concerned — very closely with his. He has, however, carried his studies considerably farther, and has shown how the different branches of the lateral-line system arise.

The general relationship noted between the auditory plate, the branchial sense-organs, and the lateral line is very similar to what Wilson found in sea bass. In that form the connection between ear, branchial organs, and lateral line is evidently more clear on account of the presence of a distinct sensory furrow and the way in which the separation into three parts takes place.

The general thickening in the sharks is not so well circumscribed. The fact that these different organs proceed from a common epithelial thickening indicates relationship of a fundamental kind. After separation the ear gives further evidence of its relationship with the lateral organs by preserving its canal (endolymphatic duct) connection with the exterior and developing in a manner that is characteristic of the canal organs of the lateral line.

Mitrophanow departs from the usual point of view that the organs of the lateral line are metameric, and in that particular, I think, I should be inclined to follow him.

The auditory plate is at first differentiated from the general thickening, and its epithelium then becomes gradually rounded up into a circular area, that is depressed in the centre — the auditory saucer. This structure sometimes covers the space of three neuromeres, and I have one surface preparation in which the outer epithelium shows three bars running vertically across it; these bars correspond with the underlying neuromeres.

We may interpret these superficial bars either as being moulded upon the underlying neural segments, or as being

due to the same general cause as the neural segments. I am of the opinion that the latter alternative is the correct one.

When first formed, the auditory saucer is opposite the ninth neuromere; but subsequently, while it is still in the saucer condition, it shifts backwards, and comes to lie at the side of the tenth neural segment, and later still, as a capsule, it lies opposite the eleventh neuromere.

The history of the auditory vesicle in sharks has been worked out beyond this period by Ayers ('92), and it is very clear from its mode of growth that it is directly related to the canal organs of the lateral line.

A consideration of the so-called branchial sense-organs and their ganglia is reserved to be published later with the part on the Nerves.

NOTE. — I have been indebted to the persons mentioned below for material that has helped fill up the gaps in my collection of embryos, and I desire here to express my appreciation of their kindness.

To Miss Julia B. Platt, for the loan of sections of *Acanthias*; to Dr. E. L. Mark, for young stages of *Squalus*; to Mr. Frank Smith, for a considerable number of embryos; to Professor J. E. Reighard for the loan and free use of his entire collection of elasmobranch embryos; to Professor A. D. Morrill, for early stages of *Squalus*.

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EXPLANATION OF FIGURES.

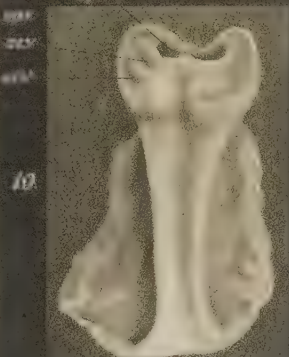
Reference Marks.

<i>1, 2, 3, 4, etc.</i>	Metameric segment.	<i>mes.</i>	mesoblast.
<i>AA'</i>	line drawn in front of the anterior neuromere of the vagus nerve.	<i>met.</i>	metameric segment.
<i>au.</i>	auditory vesicle.	<i>na.</i>	nasal epithelium.
<i>ac. v.</i>	accessory optic vesicle.	<i>op. n.</i>	optic nerve.
<i>al. c.</i>	anterior portion of alimentary canal.	<i>op. s.</i>	optic stalk.
<i>cbl.</i>	cerebellum.	<i>op. v.</i>	optic vesicle.
<i>cbr.</i>	prosencephalon.	<i>p.</i>	paraphysis.
<i>ep.</i>	epiphysis.	<i>so.</i>	serial sense-organ.
<i>H. C.</i>	mandibular head cavity.	<i>Thl.</i>	thalamencephalon.
<i>mb.</i>	mid-brain.	<i>Thl. op.</i>	thalamus opticus.
		<i>ven. 4.</i>	fourth ventricle.
		<i>Zp.</i>	Zirbelpolster.





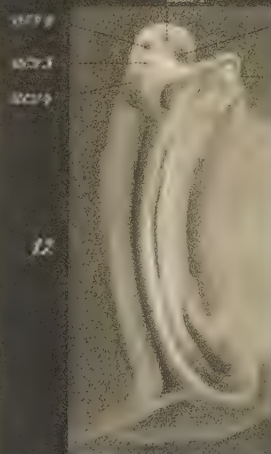
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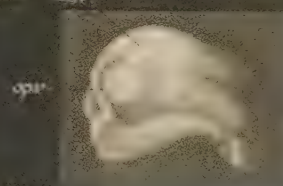
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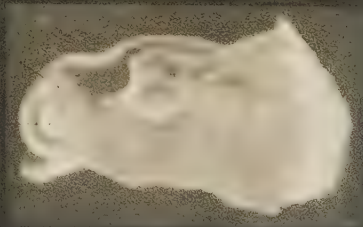


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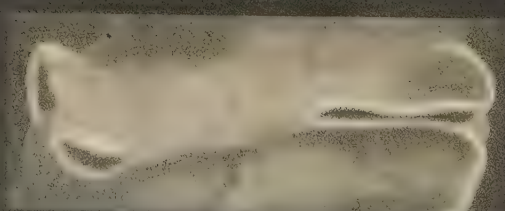
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EXPLANATION OF PLATE XXVI.

The figures are from untouched negatives and are noteworthy in showing the very early condition of the optic vesicle, the accessory vesicles and in some cases the primitive metameric segments. They are all photographs of *Squalus acanthias* and, with the exception of Fig. 7, are $\times$ about 20 diameters.

FIG. 1. Photograph of embryo between Balfour's stages *B* and *C*.

FIG. 2. Somewhat older embryo. The embryonic rim on the left side shows faintly some of the metameric segments (not reproduced by the artist).

FIG. 3. Slightly older embryo to show the formation of the head-plate and the central wedge-shaped process thereon. This is the stage in which the neural folds are started along the margins of the body. They are ventrally curved.

FIG. 4. Stage with a rounded head-plate when the optic vesicles first become evident.

FIG. 5. Another embryo, about the same age as the preceding, in which the optic vesicles and one of the accessory vesicles are shown.

FIG. 6. Somewhat older embryo showing the infolding for the optic vesicles extending across the median plane.

FIG. 7. Older embryo somewhat higher magnified. The head-plate is very broad, the trunk narrow. The neural folds of the head lie nearly in the horizontal plane. The metameric segments show well on the left margin of the head-plate. They exist in the earlier stages, but are very difficult to catch with the camera.

FIG. 8. Specimen in which the depressions for the optic vesicles show very distinctly.

FIGS. 9 and 10. Two embryos older (although smaller) than the preceding. In both two pairs of accessory optic vesicles are to be seen on the cephalic plate back of the primary optic vesicles.

FIGS. 11 and 12. Two specimens slightly older than the preceding two, seen from different point of observation.

FIG. 13. Embryo after the neural folds have begun to grow upwards, seen obliquely from the left side. Gives an external view of the vesicles on one side and an internal view of them on the opposite side of the neural folds.

FIG. 14. Embryo from which Fig. 29, Plate XXVII, is drawn. Shows metameric segments on the exposed ventral surface of the neural folds.

FIG. 15. Embryo of same age as Fig. 13 and Fig. 31, Plate XXVII. Shows metameric segments on the neural folds in front of the eye vesicles.

FIG. 16. Embryo of the same age as Fig. 9 just above it. Seen in a position more favorable to bring out the accessory vesicles on the cephalic plate. The optic vesicle on the right side shows as an external protuberance.

FIG. 17. Somewhat older embryo viewed obliquely from above. Shows the optic vesicle of the right side as an external rounded protuberance, and that of the left side from within as a cup. Behind the optic vesicle on the left side of the cephalic plate are four accessory vesicles. They show their serial relation with the primary optic vesicle.

FIG. 18. Specimen showing a large development of the central tongue-like process. Embryo slightly older than that in Fig. 7.

FIGS. 19 and 20. Side view of two heads of embryos with open neural groove to show the external appearance of the optic vesicle.

FIG. 21. Embryo of same age as Fig. 13 viewed obliquely from above.

FIG. 22. Side view of embryo of the same age (with broadly open neural groove) to show the external appearance of the optic vesicle and the other vesicles behind it.

FIG. 23. Embryo after partial closure of the neural groove. Showing two openings, an anterior and a posterior one, into the neural canal.

FIG. 24. Somewhat older specimen with head broken off. Showing three openings (smaller than those in the preceding embryo) into the neural canal.



EXPLANATION OF PLATE XXVII.

All the figures are of *Squalus acanthias*; they are drawn with the aid of the camera, and are all $\times 45$ diameters.

FIG. 25. Young embryo intermediate between Balfour's stages *B* and *C*. The embryo so far as formed is divided into eight pairs of metameres and these are continued without break, or any change in character, into the halves of the embryonic rim. The eleventh metamere which, in later stages, lies in front of the vagus nerve is now on either side the third one from the axial embryo.

FIG. 26. Somewhat older embryo, showing the change in form of the head region. The axial embryo now includes about fifteen pairs of metameres.

FIG. 27. Slightly older than the preceding; there are about eighteen pairs of metameres in the axial part of the embryo and, as in the former instances, are continued into the embryonic rim. Two longitudinal marginal furrows have appeared, separating two marginal bands from the rest of the embryo. Along the line of these furrows are seen four depressions that mark the very beginning of segmental sense-organs.

FIG. 28. View from the upper side of the same embryo illustrated in Fig. 29. The cephalic plate is now clearly marked off from the more slender trunk region. The depressions for the optic vesicles (*op.*) have made their appearance.

FIG. 29. View of the same embryo from the ventral aspect. The yolk has been completely removed, and we get a view directly into the gastrular cavity. There are eleven pairs of metameres in the broad part of the cephalic plate. The neural folds are ventrally curved. The outlines of the figure and the neural segments are too symmetrical.

FIG. 30. Older embryo with neural folds lying in the horizontal plane. The broad cephalic plate is in marked contrast with the slender trunk.

FIG. 31. Embryo in which the neural folds have nearly attained the vertical plane. The neural groove is still open. The optic vesicle (*op.*) and the combined vesicle of mid-brain and accessory optic (*mb. + A. op.*) vesicle show on the sides of the head. There is also the beginning of mandibular head cavity (*H. C.*) and the branchial pouch. The original metameric divisions are still very plain.

FIG. 32. Embryo just after the closure of the neural groove in the anterior end. The posterior part of the neural canal is not completely closed. Note the metameric divisions indicated by numbers 1, 2, 3, etc.

FIG. 33. Embryo after complete closure of the neural groove and before the appearance of the ear vesicle.

FIG. 34. Embryo after the differentiation of the ear saucer. The five anterior metameric divisions are no longer distinguishable, those of the hind-brain are prominent and are approximated in the middle plane. One gill-cleft has broken through. The nasal pit has started.

FIG. 35. Slightly older embryo showing several characteristic changes. The line of neural segments are being forced apart by lateral growth of the roof of the hind-brain. The fifth nerve is plainly visible. Over the gill-clefts runs a continuous nodulated thickening containing the branchial sense-organs and the rudiment of the lateral line.

FIG. 36. Somewhat older embryo differing from the preceding mainly in showing the rudiments of the seventh, eighth, ninth, and tenth nerves. Note the lens and choroid fissure in the eye vesicle.

FIG. 37. Slightly older than the preceding. The line of neural segments are undergoing some changes whereby the concavity on the lower margin is made to correspond with a crest on the upper margin. In embryos of about the age represented in Fig. 36 or a very little older the epiphysial outgrowth arises from the roof of the thalamencephalon.

EXPLANATION OF PLATE XXVIII.

A series of partial dissections of embryos of *Squalus* in such a way that the brain-walls have been laid bare. Figs. 46-49 $\times 45$ diameters. Figs. 50-56 $\times 10$ diameters.

FIG. 38. Embryo with open neural groove. The epidermal layer and the mesoderm have been removed from the sides of the brain-wall; behind the optic vesicle is seen a bilobed protuberance—the combined mid-brain vesicle and the anterior accessory optic vesicle.

FIG. 39. Older embryo with neural canal partly formed. The specimen shows the same condition of mid-brain vesicle and that of the anterior accessory optic vesicle.

FIG. 40. The brain-walls of an embryo of the same age as that shown in Fig. 32.

FIG. 41. Embryo before dissection, showing especially well the contours of the brain-walls.

FIG. 42. The same embryo after exposure of the brain-walls by dissection. The thalamencephalon is well exhibited. The mid-brain is bilobed.

FIG. 43. Sketch of partially dissected embryo just after the appearance of the auditory vesicle.

FIG. 44. The exposed brain-walls of an embryo slightly older than that represented in Fig. 34.

FIG. 45. Brain of embryo about same age as that in Fig. 35. The auditory vesicle has been left in position. The mid-brain is now indistinctly trilobed.

FIG. 46. Brain-walls of embryo with the optic vesicle removed. About the same age as the preceding.

FIG. 47. Dissection of the brain of the embryo of which Fig. 36 is an external view. The mid-brain is distinctly trilobed. There are eight clearly marked segments in the hind-brain.

FIG. 48. Dissection of embryo slightly older than the one represented in Fig. 37. The thalamencephalon is now definitely marked out by furrows; it bears upon its summit two rounded confluent protuberances.

FIG. 49. Dissection of brain of embryo somewhat older than the preceding. The thalamencephalon is clearly defined. The posterior protuberance from its roof has grown much faster than the anterior one. The former is the beginning of epiphysis. There are nine neural segments in the hind-brain.

The embryos are now too large to represent advantageously on the same scale, and in the following figures the scale of magnification is reduced from 45 to 10 diameters.

FIG. 50. Shows embryo of same age as that represented in Fig. 32.

FIG. 51. Embryo of nearly the same age as that represented in Fig. 34.

FIG. 52. Partly dissected embryo of about the same age as that represented in Fig. 36 and again in Fig. 47.

FIG. 53. Brain-walls of an embryo just older than that shown in Fig. 49.

FIGS. 54 and 55. Successively older embryos to show especially the changes in the thalamencephalon and the outgrowth from its roof of the epiphysis.

FIG. 56. The same brain shown in Fig. 55, with the cerebral lobes removed and turned so as to view directly against the epiphysis.



EXPLANATION OF PLATE XXIX.

Figs. 57-62 a continuation of the series of dissections shown on the two previous plates. All $\times 10$ diameters. Figs. 65-87 $\times 45$ diameters.

FIG. 57. Side view of brain of an older embryo than that sketched in the foregoing figure. The neural segments have now become obliterated, and the three lobes of the mid-brain (secondary divisions) are no longer distinguishable. The epiphysis is well developed; in front of it is a semicircular fold of the brain-wall; this structure is the remnant of the elevation which started in front of the epiphysis on the roof of the thalamencephalon (see Figs. 48 and 49). It has become reduced by compression between the rapidly growing adjacent brain regions. The paraphysis is also visible in this drawing.

FIG. 58. View of the same brain from above looking into the cavity of the fourth ventricle.

FIG. 59. The same brain after removal of the cerebral lobes and arranged so as to show the epiphysis in front view.

FIG. 60. Brain of older embryo showing substantially the same features as the preceding.

FIG. 61. The cerebral lobes of the same brain after removal. Note the paraphysis arising from the posterior part of the roof of the prosencephalon.

FIG. 62. The same brain after removal of prosencephalon and arranged so as to show to best advantage the epiphysis with its stalk.

FIG. 63. Horizontal section of embryo shown in Fig. 25 to show the general appearance of the metameres in section. $\times 50$.

FIG. 64. Horizontal section of embryo sketched in Fig. 26 showing metameres in section, and adjacent layer of mesoderm. $\times 50$.

FIG. 65. Section of head of *Torpedo ocellata* through the optic vesicles at the time of their first appearance. Compare this with Zieglers' Pl. IV, Fig. 19¹.

FIGS. 66, 67, 68, 69, 70. Successive sagittal sections of embryo just after closure of the neural groove and prior to the appearance of the ear vesicle. Show the primary fore-brain, the mid- and hind-brains, and three very prominent neural segments of the hind-brain. The segments are the seventh, eighth, and ninth respectively. When the ear is first differentiated it arises opposite the ninth segment.

FIG. 71. Deeper section of the same embryo showing a curved line of mesoderm over the mandibular cavity.

FIG. 72. Sagittal section of embryo near the median plane after the formation of the auditory saucer and the complete closure of the neural groove. The five neural segments of the fore- and mid-brain are exhibited. The second neural segment nearly coincides in position with the neuropore.

FIGS. 73, 74, 75. Three successive sagittal sections of an embryo of about the age of that drawn in Fig. 34. It is slightly older, shows well the neuromeres of the hind-brain. The ear capsule is in the space of the tenth neuromere. In front of it in Figs. 73 and 74 are seen the roots of the seventh and eighth nerves.

FIGS. 76-87. Sagittal sections of the specimen photographed as Fig. 17, Pl. XXVI. Giving evidence of a series of cup-like depressions on the neural plate of head and trunk. The series of these cupped areas is terminated in front by the optic vesicles. On the cephalic plate they are relatively large and resemble the primary optic vesicles in mode of origin and in structure. How far the series extends into the trunk I have not been able to determine.



EXPLANATION OF PLATE XXX.

FIGS. 88-112. Twenty-five transverse sections of an embryo very slightly older than that represented in Fig. 16. The sections 88-100 lie in the region of the cephalic plate. The following sections 105-112 lie in the neck and trunk regions.

These sections are remarkable in bringing to light serial depressions along the walls of the neural folds. They show that the serial cup-like differentiations extend back of the cephalic plate. I have not been able to determine the number of serial differentiations of this character in the embryo, but it is clear there are several pairs behind the cephalic plate upon which I have noted in surface study four pairs in addition to the eyes. $\times 45$ diameters.

[Figs. 113-115 were drawn from nature by Miss Tanetta Gilleland. Figs. 117 and 118 are taken from Kupffer's "Studien zur vergleichenden Entwicklungsgeschichte des Kopfes der Kranioten," and Fig. 116 is after Froriep.]

FIG. 113. View of the head-end of embryo of *Amblystoma* in the open neural groove stage, $\times$ about 10 diameters. Shows segmental folds in the neural folds and a smooth neural plate.

FIG. 114. View on the caudal extremity of the same embryo.

FIG. 115. Embryo of *Rana palustris* showing large obvious segmental folds in the median plate and also smaller fainter folds in the neural ridges. The latter correspond to the segments in the neural ridges in *Amblystoma*.

FIG. 116. Embryo of *Triton cristatus* after Froriep. Showing large obvious folds in the median plate with unsegmented neural ridges. These median folds probably correspond to those in *Rana palustris* and not to the metameric divisions in the neural folds.

FIG. 117. View on the head-end of *Salamandra atra*, according to Kupffer, showing segmental folds in the median plate but none in the neural ridges. Compare with Fig. 115.

FIG. 118. Caudal end of same embryo.

Figs. 119-124 are sagittal sections of *Squalus acanthias*. $\times$ about 45 diameters.

FIG. 119. Section of head of embryo about same age as those represented in Figs. 37 and 48, showing the roof of the thalamencephalon raised into two elevations.

FIG. 120. Somewhat older embryo showing the tubular-like growth of the epiphysis.

FIG. 121. Still older stage showing reduction of the anterior part of roof of thalamencephalon and great increase in depth of the furrow in front of the thalamencephalon.

FIG. 122. Older stage in which the paraphysis has made its appearance from the roof of the prosencephalon. The choroid plexus has also started.

Figs. 123 and 124. Two older stages showing the increase in length of the epiphysis; its distal end is somewhat enlarged and is inserted into a concavity in the cranial roof. The paraphysis is indicated at *pp.*—The choroid plexus is considerably increased in extent and hangs into the cavity of the fore-brain.

